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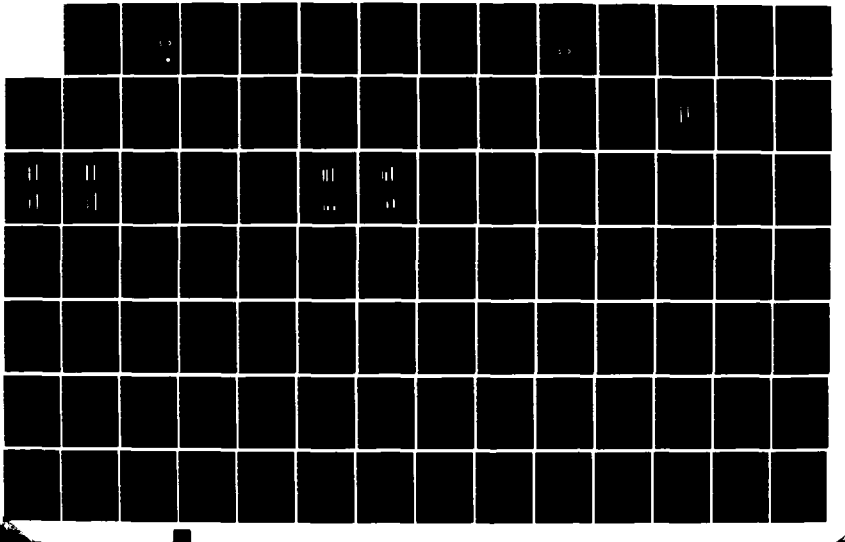

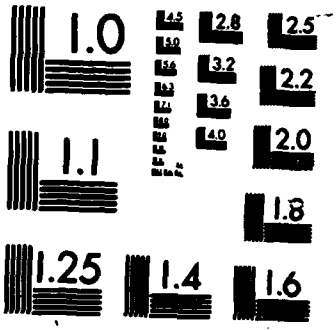

Report USAFSAM-TR-84-31

(2)

EFFECTS OF LONG-TERM LOW-LEVEL RADIOFREQUENCY RADIATION EXPOSURE ON RATS

VOLUME 7. METABOLISM, GROWTH, AND DEVELOPMENT

Robert B. Johnson, B.S.
Lawrence L. Kunz, D.V.M.
Desmond Thompson, M.S.
John Crowley, Ph.D.
Chung-Kwang Chou, Ph.D.
Arthur W. Guy, Ph.D.

Bioelectromagnetics Research Laboratory
Department of Rehabilitation Medicine
School of Medicine
University of Washington
Seattle, WA 98195

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USAF SCHOOL OF AEROSPACE MEDICINE
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas 78235-5000

NOTICES

This final report was submitted by the Bioelectromagnetics Research Laboratory, Department of Rehabilitation Medicine, School of Medicine, University of Washington, Seattle, Washington 98195, under contract F33615-80-C-0612, job order 7757-01-71, with the USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks AFB, Texas. Dr. Jerome H. Krupp (USAFSAM/RZP) was the Laboratory Project Scientist-in-Charge.

When government drawings, specifications, or other data are used for any purpose other than in connection with a definitely Government-related procurement, the United States Government incurs no responsibility nor any obligation whatsoever. The fact that the Government may have formulated or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication, or otherwise in any manner construed, as licensing the holder, or any other person or corporation; or as conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

JEROME H. KRUPP, D.V.M.
Project Scientist

JOHN C. MITCHELL, B.S.
Supervisor

ROYCE MOSER, Jr.
Colonel, USAF, MC
Commander

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For 25 months 100 male SPF rats were exposed to pulsed 2450-MHz circularly polarized microwaves at an average power density of .480 mW/cm^2; 100 rats served as sham-exposed controls. Analysis of daily body weight and food and water consumption failed to distinguish the treatment groups. Periodic assessment of oxygen consumption, carbon dioxide production, and respiratory quotient also failed to reliably differentiate the groups. Whole-body carcass analyses, including organ mass, fatty acid profile, and mineral content, were conducted on 20 rats killed after 13 months exposure and 20 killed at termination of the project. With one exception, these measures did not indicate significant differences between the treatment groups. A 75% increase in adrenal mass was shown for the exposed animals at the termination kill. When the animals with adrenal tumors were excluded, there was no statistical significant difference between the exposed and sham groups. Respiratory gas exchange measures were obtained from an additional 36 young male rats during a subsequent 3-month period of exposure. No consistent significant effects were observed in these young animals. Originator-Supplied Keywords include:

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Jerome H. Krupp, D.V.M.	(512) 536-2576	USAFSAM/RZP

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EFFECTS OF LONG-TERM LOW-LEVEL RADIOFREQUENCY RADIATION EXPOSURE ON RATS

VOLUME 7: METABOLISM, GROWTH, AND DEVELOPMENT

INTRODUCTION

Although more than 6000 articles on the biological effects of electromagnetic radiation have been published, whether or not long-term low-level exposure to radiofrequency radiation (RFR) represents a human health hazard remains unclear (Czerski et al., 1974; Glaser and Dodge, 1977; Tyler, 1975; Justesen and Guy, 1977; Justesen and Baird, 1979; Gandhi, 1980). Most exposure protocols completed to date have been of relatively short duration and restricted sample size, thus providing little insight into cumulative effects.

During the past three years, the Bioelectromagnetics Research Laboratory at the University of Washington conducted the largest single evaluative study of the bioeffects from microwaves ever undertaken. Sponsored under a U.S. Air Force School of Aerospace Medicine (USAFSAM) contract, the goal of the project was to investigate purported adverse effects on health after long-term exposure to pulsed microwave radiation. The major emphasis was to expose a large population of experimental animals to microwave radiation throughout their lifetimes and monitor them for cumulative effects on general health and longevity.

This technical report is Volume 7 in a series covering each of the major subtopics of the chronic-exposure study. It presents a detailed description of the methods and results of the daily measures of body mass, food and water consumption, and respiratory gas exchange, and of the whole-body analyses conducted after 13 and 25 months of exposure.

The first three volumes covered in detail the exposure facility, animal maintenance procedures, and various dosimetry studies. A brief summary of these topics follows.

A unique exposure facility was prepared that allowed 200 rats to be maintained under specific-pathogen-free (SPF) conditions while housed in individual, circularly polarized waveguides. The exposure facility consisted of two rooms, each containing 50 active and 50 sham-exposure waveguides to house subjects. Each room contained two 2450-MHz pulsed microwave generators, each capable of delivering a maximum of 10 W average power at 800 pps with a 10-μsec pulse width. This carrier was square-wave modulated at an 8-Hz rate. The power-distribution system delivered .144 W to each exposure waveguide for an average-power density of .48 mW/cm^2. Whole-body calorimetry, thermographic analysis, and power-meter reading analysis indicated that these exposure conditions resulted in average specific absorption rates (SARs) ranging from approximately 0.4 W/kg for a 200-g rat to 0.15 W/kg for an 800-g rat.

Two hundred male rats at 3 weeks of age were obtained from a commercial, barrier-reared colony and randomly assigned to exposed and sham-exposed treatment conditions. Exposure began at 8 weeks of age and continued for 25 months. Throughout this period all surviving animals were bled at regular intervals, and blood samples were analyzed for a panel of serum chemistries, hematological values, protein electrophoresis patterns, and thyroxine (T_4) and serum corticosterone levels. In addition to daily measures of body mass and food and water consumption, oxygen consumption and carbon dioxide production were periodically measured on a subpopulation of the exposed and sham-exposed groups. At regular intervals throughout the study, activity was assessed in an open-field apparatus. After 13 months, 10 rats from each treatment group were killed for immunological competence testing, whole-body analysis, and gross and histopathological examinations. The surviving 23 rats were killed at the end of 25 months, and similar analyses were made of 10 from each group.

BODY MASS AND CONSUMPTION OF FOOD AND WATER

In principle, two distinct actions of microwave radiation on mammals can be distinguished: 1) the hypothesized direct effects on biomolecules and cell structure through as yet undemonstrated mechanisms and 2) the inescapable and demonstrable thermal consequences of heat produced within biological material as a result of microwave energy absorption. The exposure levels used in this study were decisively below those known to produce obvious thermal damage to cellular processes. The intended purpose of daily measurements of body mass and consumption of food and water was to monitor endpoints that might reflect cumulative consequences of minor microwave energy absorption.

Sacher has proposed (1980, personal communication) that one possible cumulative effect of minor thermal input would be a prolongation of life to some degree and a reduction of cancer incidence in the exposed population (cf. Sacher, 1977). The probability of these occurring would depend on how the average exposed animal "metabolized" the small amount of thermal energy deposited within it. One option for an exposed animal would be to integrate the absorbed energy within its total energy budget required to maintain thermal equilibrium with the ambient environment. Integration would lessen the demand on internal chemical stores of energy. The lowered requirement for chemical energy might then result in lowered food consumption, with the potential for associated effects on total body mass and/or total fat stores. Experimentally imposed reduction of food intake (restriction) has been shown to be one of the most effective methods for enhancing longevity of the rat (McCay et al., 1935; Berg and Simms, 1960; Masoro et al., 1982).

Thermoneutrality has been defined as the ambient temperature range in which the minimum level of metabolic activity is observed. For the rat, the range has been determined to be 28-33^{o}C (Herrington, 1940). At temperatures below thermoneutrality, metabolism rises as a consequence of increased heat production. At temperatures above thermoneutrality, metabolism rises as a result of increasing motor activity associated with behavioral thermoregulation. The food intake of the rat varies as a linear

function of ambient temperature; intake is highest at ambient temperatures near $0^{o}C$ and decreases linearly to a minimum (Brobeck, 1948; Hamilton, 1967; Jakubczak, 1976).

When ambient temperature was varied and food intake monitored in rats exposed to microwaves in circular waveguides in previous studies conducted in this laboratory, food intake decreased as an additive function of both rising ambient temperature (21-$26^{o}C$) and increasing SAR (0.8-7.2 W/kg) (Moe et al., 1977; Lovely et al., 1977; Johnson et al., 1981).

In this study a maximum of .4 W/kg thermal input was to be imposed on the exposed animals, so we concluded that an environmental temperature well below thermoneutrality (e.g., $21^{o}C$) would provide the most suitable metabolic baseline for assessment of the effects of this thermal input. Within this range, the rat's thermoregulatory responses are limited primarily to variations in metabolic heat production. If the more usual vivarium conditions were used (e.g., $25^{o}C$), the rats in both treatment groups would be in a borderline environment, at the threshold of which more dramatic physiological and behavioral attempts to thermoregulate might occur, such as reduced activity (Hainsworth, 1967), saliva spreading (Hainsworth, 1968), and vasodilation of the tail (Hellstrom, 1975; Rand et al., 1965). Under such conditions the small amount of microwave energy absorbed by the exposed animals might cause them to perceive the ambient environment quite differently than would the sham-exposed animals.

Method

The animal population, housing facilities, and maintenance procedures were described in detail in Volume 1 of this series of reports. The information pertaining most directly to metabolism is summarized in the following section.

At 21 days of age, 200 male SPF rats (Sprague-Dawley) were obtained from a commercial supplier (Camm (SD) BR, Camm Research Labs, Wayne, NJ) and housed individually throughout the study in a barrier facility. For approximately 21 h/day these animals were in cylindrical Plexiglas cages placed inside wiremesh waveguide apparatus; 100 animals were maintained

under microwave-exposure and 100 under sham-exposure conditions. Each morning all animals were removed from these cages at staggered times over a 2-h period, weighed, and placed in standard filter-bonneted Plexiglas cages while their waveguide cages were being cleaned. The facility was maintained at approximately 21°C and 55% relative humidity. Fluorescent room lighting was maintained on a 12/12 light/dark cycle (lights on 0700-1900).

During the 21 h/day that the animals were housed in the waveguide apparatus, they had ad libitum access to autoclaved pelleted chow (Certified Autoclavable Rodent Chow #5014, Ralston Purina Co., St. Louis, MO) and distilled water. During data analysis, food and water intakes were not expressed as a function of body weight (cf. Wirtshafter and Asin, 1982); instead, an analysis of covariance was used to determine dependence of food/water consumption on body mass.

Results

Daily monitoring of temperatures indicated that the average temperature monitored at the midline of each alcove was 21.1°C with a standard deviation of 0.6°C, calculated on a monthly basis. The microenvironment of an animal cage can be quite different from the ambient conditions surrounding it (Murakami, 1971), so the differences in internal cage temperature were monitored at various positions within each alcove. The vertical temperature gradients within an alcove, each taken as the average of all cages on one row, measured over a 10-day period are summarized in Table 1.

Internal cage temperatures did not differ between exposed and sham-exposed animals on any row; none of the pairwise t-test comparisons are significant at the $p < .05$ level. The average alcove temperature during the measurement period was 21.20°C $\pm$ .15°C SE, and the average cage temperature was approximately 0.2-1.6°C warmer depending on vertical position within the alcove. A one-way analysis of variance showed no significant differences in temperature among the bottom three rows; however, the cages on the top row of the alcove were significantly cooler than those on the 2d row ($t = -3.02$, $p < .005$, $df = 51$). This temperature

gradient is due to the downward laminar airflow within each alcove. Fresh air enters the alcove at the top and is warmed by the animals before it is withdrawn at the bottom of the alcove.

TABLE 1. INTERNAL CAGE TEMPERATURES IN EXPOSED AND SHAM-EXPOSED CAGES WITHIN ALCOVES.

(Average temperature at center of alcoves was 21.20°C ± .15°C SE)

	Exposed	Sham	Combined[a]
Top row	21.39 ± 0.32[b]	21.94 ± 0.35	21.74 ± 0.25
Second row	22.65 ± 0.27	22.72 ± 0.24	22.68 ± 0.18
Third row	22.37 ± 0.21	22.63 ± 0.28	22.49 ± 0.17
Bottom row	22.87 ± 0.23	22.69 ± 0.20	22.76 ± 0.16

[a] Temperatures independent of treatment condition.
[b] Mean ± standard error.

At the beginning of the study, the animals were housed in their respective waveguide cages but no waveguides were energized. This provided all animals a period of waveguide adaptation under identical nonexposure conditions. At the start of this adaptation period, the animals were randomly assigned to exposed and sham-exposed conditions in a manner that roughly equated mean body mass at approximately 150 g. During the following 3 weeks, all animals lived under similar conditions, learned to obtain food and water in the waveguide/cage apparatus, and adapted to the daily handling procedure. The mean body mass and average daily food and water consumption of the exposed and sham-exposed groups for the final 3 days of the adaptation period are presented in Table 2. At the end of adaptation, exposure-group animals were 6 g heavier than the sham-exposure group (t = 1.95, p = .055, df = 198). Food and water consumption did not differ significantly between the two groups. After the 3-week adaptation period, microwave power was applied only to the exposure waveguides.

TABLE 2. BODY WEIGHT AND FOOD AND WATER INTAKE FOR 3-DAY PERIOD IMMEDIATELY PRECEDING INITIATION OF MICROWAVE EXPOSURE

	Exposed	Sham Exposed
Body mass (g)	285.4 ± 2.1[a]	279.6 ± 2.1
Food intake (g)	26.5 ± 0.5	26.2 ± 0.5
Water intake (g)	32.2 ± 0.8	32.9 ± 0.9

[a]Mean ± SE.

A summary of average weekly body mass for the 25-month study is presented in Fig. 1. No overall differences in body mass are seen between treatment groups. Both exposed and sham-exposed animals grew rapidly during the first few months, then gradually approached an asymptotic level. During the final months a reduction in body mass in both groups reflected weight loss due to aging and disease processes.

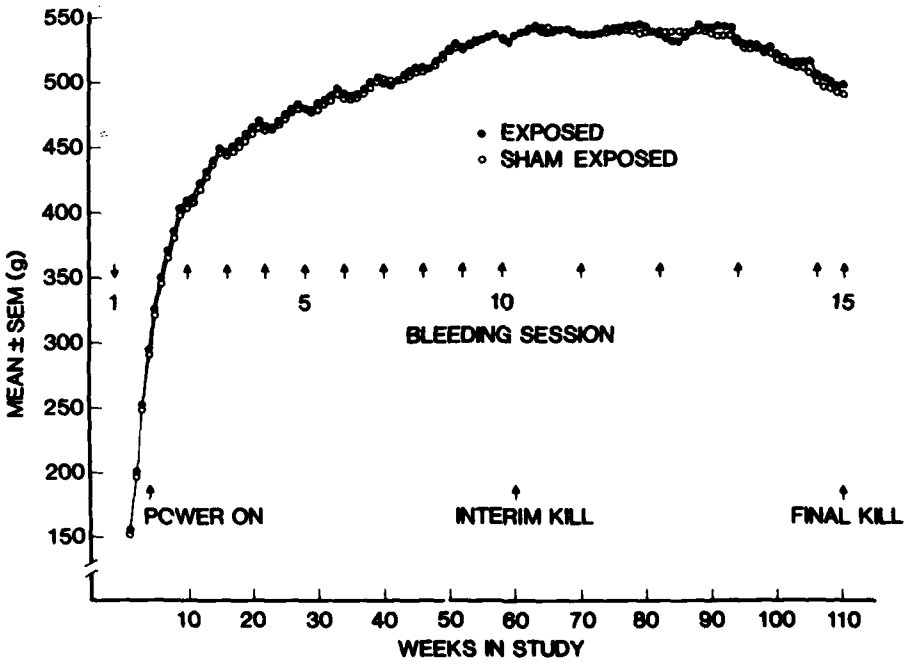

Figure 1. Mean weekly BODY MASS throughout 25-month study. Arrows indicate periodic bleeding sessions as well as other significant events during the course of the study.

Mean daily food and water consumption, calculated weekly throughout the study, are presented in Figs. 2 and 3. Again, no overall differences are apparent between treatment groups in either of these measures.

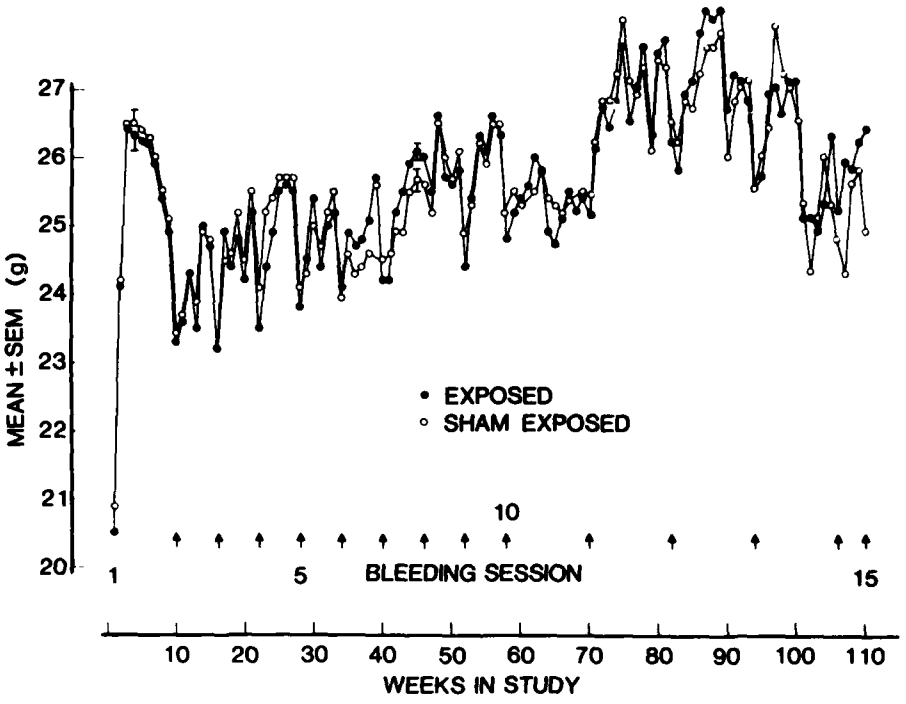

Figure 2. Mean daily FOOD CONSUMPTION throughout 25-month study. Arrows indicate significant events.

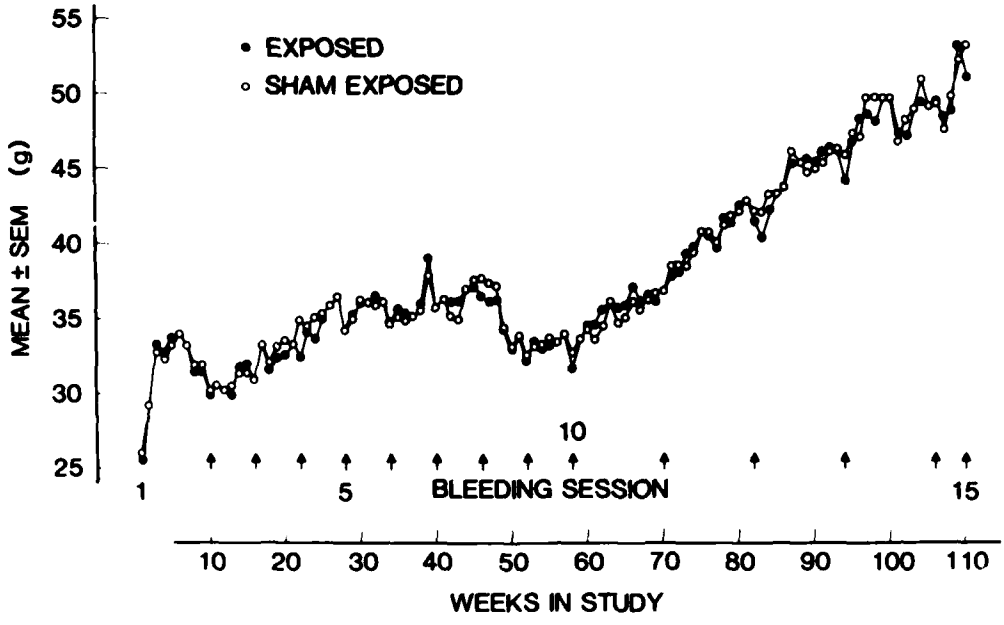

Figure 3. Mean daily WATER CONSUMPTION throughout 25-month study. Arrows indicate significant events.

In these figures the shape of the curve for both groups of animals is very regular throughout the adaptation period and the first 6 weeks of exposure. Coincident with blood sampling session #2, however, the weekly increment in body mass of all animals is perceptibly disturbed. Similar disturbances, with varying severity, are coincident with each blood sampling session throughout the study. These disturbances generate a scalloped or saw-toothed pattern in the plot of body mass independent of treatment condition. A similar pattern occurs to a lesser degree in the plots of food and water consumption.

In Fig. 4 we repeat a portion of the mean weekly data on body mass from Fig. 1, starting after the rapid growth in the adolescent rat and using a scale that makes the scalloped pattern more apparent.

Before we could make a meaningful statistical analysis of body mass and food and water consumption, a format was devised to synchronize the data within each blood-sampling week with the specific date an animal was bled. This step was necessary because blood was sampled over a 4-day period during the week. If we simply averaged the data on a weekly basis, some specific information concerning the impact of blood sampling on measurements of body mass and food and water consumption would be lost.

A representative compilation of the changes in body mass and consumption of food and water during the days immediately following a blood sampling is presented in Fig. 5. These data are from session #2, which was the first blood sampling after microwave exposure was started. Day-0 data were collected just before an animal was bled and therefore represent a stable comparison point for all animals prior to initiation of the blood sampling procedures.

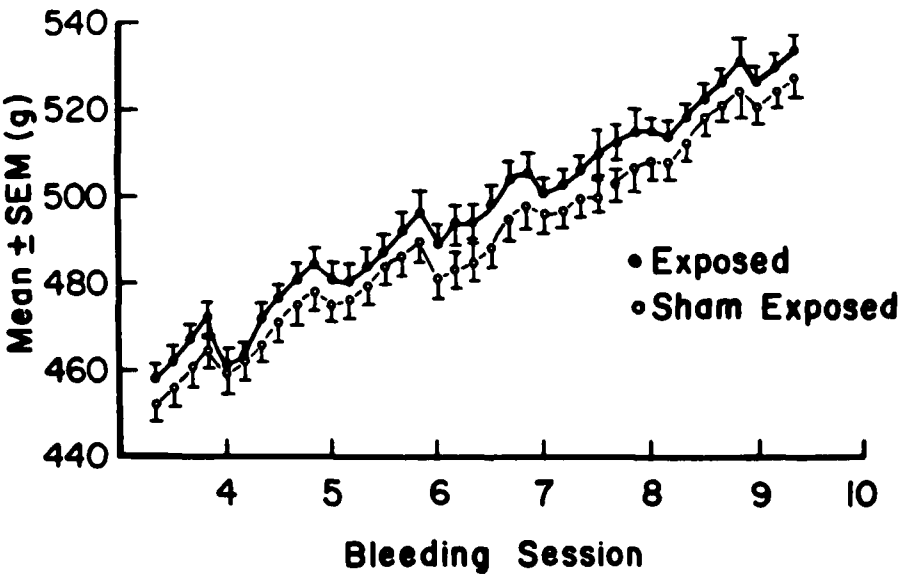

Figure 4. Expanded-scale presentation of BODY MASS emphasizing periodic decrements coincident with regular bleeding sessions. Session #4 corresponds to 22 weeks in study (compare Fig. 1).

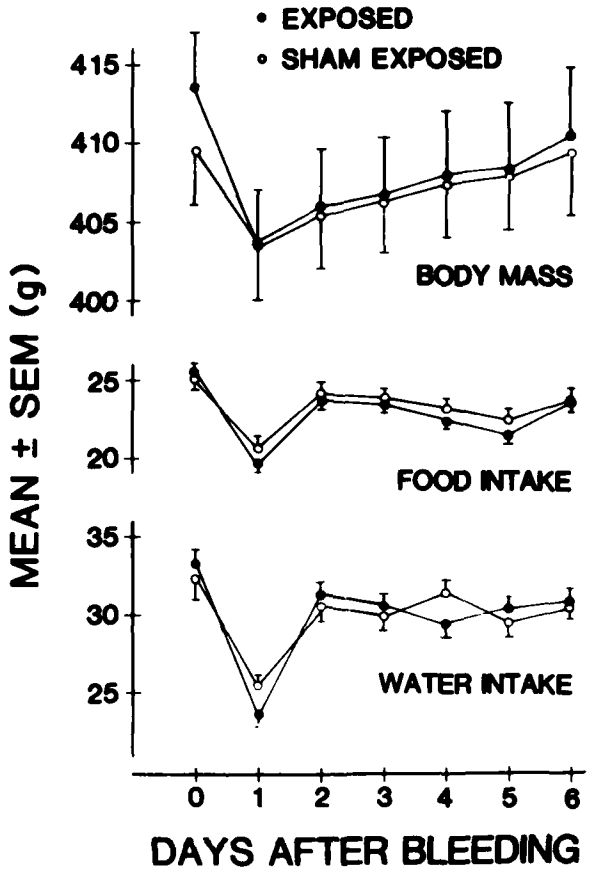

Figure 5. Sequenced measurements of BODY MASS and FOOD and WATER CONSUMPTION during week of second bleeding session.

A larger relative loss of body mass is seen for the exposed animals in the first-day response to blood sampling, but similar reductions for food and water consumption are seen for both groups. A summary of the analysis of the first-day reduction of body mass (i.e., Day #1 - Day #0) is presented in Table 3. In 10 of the subsequent 12 blood samplings, relatively larger reductions in body mass are apparent on the first day; however, none of the pairwise t-test comparisons of body mass reduction show statistically significant differences.

TABLE 3. ANALYSIS OF MEAN BODY-MASS LOSS (g) ON FIRST DAY AFTER EACH BLOOD SAMPLING

	Exposed			Sham					
Session	M	SEM	N	M	SEM	N	t	p	df
2	8.76	1.43	60	6.13	1.24	62	1.39	0.17	120
3	8.20	1.18	72	6.89	1.07	75	0.82	0.41	145
4	8.42	1.09	77	7.34	1.30	78	0.64	0.52	153
5	7.88	1.16	88	7.19	1.09	86	0.43	0.67	172
6	9.55	0.98	93	8.24	1.15	86	0.87	0.38	177
7	6.30	1.69	50	5.74	1.35	41	0.26	0.80	89
8	2.55	0.74	90	2.91	0.94	83	-0.30	0.76	171
9	6.12	1.11	52	5.71	1.22	44	0.25	0.81	94
10	6.28	0.93	79	6.16	1.32	74	0.08	0.94	151
11	6.67	1.34	61	4.96	0.98	63	1.14	0.26	122
12	5.78	1.52	49	3.84	0.85	51	1.12	0.27	98
13	6.34	2.19	32	4.78	1.82	33	0.55	0.58	63
14	5.35	1.38	16	5.69	0.82	17	-0.21	0.83	31

Assuming that food intake would vary as a function of body size, we analyzed food consumption for the first-day response to blood sampling as an analysis of covariance involving food consumption and body mass. This analysis indicated no differences in first-day food intake between treatment groups throughout the 13 blood-sampling sessions considered. The analysis indicated, however, that the body mass coefficient was significantly different from zero; generally, as body mass increased by 1 g, food consumption increased by .04 g.

Also, recovery of body mass during the week following blood sampling was examined as the difference in body mass between the last day of the week and the day of the blood sampling. Table 4 presents a summary of this analysis; a positive value indicates weight regained in excess of body mass at the start of blood sampling. In 12 of the 14 sessions the exposed animals apparently regained more weight by the sixth day after blood sampling, but none of the pairwise t-test analyses show significant differences.

TABLE 4. ANALYSIS OF MEAN BODY-MASS RECOVERY (g) DURING THE WEEK AFTER EACH BLOOD SAMPLING

	Exposed			Sham					
Session	M	SEM	N	M	SEM	N	t	p	df
2	1.70	2.54	60	0.18	1.83	62	0.49	0.63	120
3	5.04	1.54	71	7.54	1.99	75	-0.99	0.32	144
4	6.51	1.77	77	3.35	1.78	78	1.26	0.21	153
5	5.06	1.36	88	4.71	1.51	86	0.18	0.86	172
6	8.81	1.60	93	8.47	2.05	86	0.13	0.89	177
7	6.37	3.46	44	1.49	1.67	38	1.21	0.21	80
8	2.31	0.82	90	1.16	1.24	83	0.78	0.43	171
9	4.14	1.32	42	3.75	1.62	38	0.19	0.85	78
10	6.80	1.31	78	7.77	2.37	74	-0.36	0.72	150
11	5.16	1.61	60	4.81	1.24	62	0.17	0.86	120
12	6.89	1.95	48	3.42	1.23	51	1.52	0.13	97
13	5.02	5.85	31	3.24	2.01	32	1.90	0.06	61
14	7.99	1.74	16	7.99	2.53	16	0.00	0.99	30

We also used growth-curve analysis to investigate the recovery period, using the method of Khatri as described in Morrison (1976). We compared the growth curves for the treatment groups during the week following each blood sampling but found no significant differences. Quadratic terms were included, but in all instances the coefficients were zero. We also examined the curves for trend over time and found no systematic pattern.

Discussion

Growth curves for microwave and sham-exposed animals throughout this study are in general agreement with that reported for the Sprague-Dawley rat (Berg, 1960; Masoro, 1980). The asymptotic body mass approached may have been somewhat lower than expected, possibly owing to the periodic "stunting" effect coincident with the start of the regular blood-sampling sessions.

The average daily food intake of approximately 25-26 g is higher than usually reported for the rat and indicated by the feed manufacturer (12-15 g/day). These "normal" values, however, are for animals housed in a standard animal facility maintained at a higher ambient temperature (25°C). The amount of food eaten by the animals in our facility is in consonance

with that reported for animals housed at lower ambient temperatures (Brobeck, 1948; Hamilton, 1963; Jakubczak, 1976) and in previous studies that used the waveguide apparatus (Moe et al., 1977; Lovely et al., 1977, 1978; Johnson et al., 1981).

The regularly occurring decrements in body mass and food and water consumption with each blood sampling is not an altogether unexpected result. In previous studies using this type of waveguide apparatus, food consumption dropped markedly immediately after anesthetization for blood sampling and could remain depressed for up to a week. This reduction has been interpreted as an indicator of the stressful nature of the anesthetization procedure, independent of any other treatment conditions that may be imposed on the animals (Lovely et al., 1978). In the waveguide apparatus, an animal must, in one sense, work to get his daily ration of food; i.e., food is not lying on the floor, it must be eaten through the opening of the food chute. The reduced food consumption could also be attributed to reduced vigor in the days immediately after the anesthetization.

The 2 ml of blood removed from these animals represents a maximum of 15% of total blood volume for young animals and approximately 5% for mature animals (Bruckner-Kardoss and Westman, 1974; Yale and Torhorst, 1972). This volume is well below the critical level of blood loss that causes death; however, the recurring perturbations of body mass and food and water consumption suggest that this blood loss or the associated anesthetization and blood sampling procedures are significant stressors.

The similarity in overall patterns of growth, food and water consumption, and body-mass loss and recovery responses for the exposed and sham-exposed populations indicates that no cumulative effects of microwave energy absorption were apparent at this level of exposure and using these measures of long-term energy balance.

WHOLE-BODY COMPOSITION

As part of the data base for an overall analysis of metabolic energy utilization, whole-body carcass analyses were completed of animals killed after 13 and 25 months of exposure as part of interim and final histopathological screening procedures. At 13 months, 10 exposed and 10 sham-exposed animals were randomly removed from the study and killed; and at the end of 25 months, all surviving animals, 12 exposed and 11 sham exposed, were killed. The masses of various organs, general carcass composition, fatty acid profile, and mineral content were determined. These analyses can be useful for detecting changes in body composition that may have resulted from cumulative differences in energy metabolism between the exposed and sham-exposed animals. The methods and results of these measurements are described in the following sections.

General Procedure

On the morning of the kill the animals were removed from their waveguide cages, weighed, anesthetized with a Halothane-nitrous oxide/oxygen mixture, and then exsanguinated via the brachial artery. As the various organs were removed from the carcass (prior to tissue sampling for histological purposes), they were each weighed on an electronic analytic balance with an accuracy of $\pm$ .001 g. Once all representative tissue samples had been collected, the eviscerated carcass was frozen for later analysis of constituents.

Organ Mass

The following organs were weighed after removal: heart, brain, liver, left and right kidneys, left and right testicles, and left and right adrenals.

The mean body masses for the exposed and sham-exposed animals killed in the interim and final determinations are compared in Fig. 6. The small differences in mean body mass are not significant at either time.

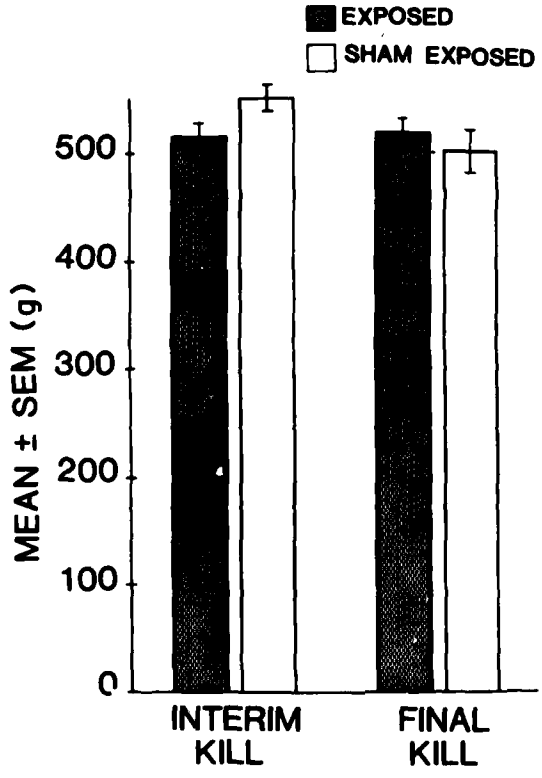

Figure 6. BODY MASS of exposed and sham-exposed animals.

Generally, an analysis of organ mass might proceed on the assumption that organ mass is highly correlated with total body mass and therefore must be proportioned with respect to body mass prior to statistical analysis. Intuitively, this assumption makes sense in the extreme; organs from a 600-g adult rat will normally be of greater mass than those from a 100-g juvenile animal. The validity of this technique, however, has not always been demonstrated satisfactorily. For example, Ray et al.(1968) have shown that expressing organ mass as a function of body mass can account for sex-dependent but not temperature-dependent variations in the organ mass of rats.

This proportioning was not completed because of arguments that the practice can lead to questionable conclusions (Wirtshafter and Asin, 1982). Instead, we used an analysis of covariance for each organ, with body mass as a covariate. A significant effect of body mass showed for only two organs, brain and liver. Since the conclusions drawn from the covariance analysis did not differ from those derived from a separate analysis of variance performed on the raw data, all data presented will be in their original form. Mean organ mass for liver, heart, and brain, and total mass for kidney, testicles, and adrenals are presented in Figs. 7 through 12, respectively, for the exposed and sham-exposed groups.

The organ-mass analysis for both treatment groups is summarized in Table 5. The table contains individual t-test results for each organ comparison during the interim and final kills, also a summary of the two-way analysis of the interim/final kill comparisons. The pairwise comparisons for treatment groups, at either interim or final kill, are significant for only the testicles and adrenals. Testicular mass reflects a marginally significant difference at the time of the interim but not the final kill; and adrenal mass, a highly significant difference at the time of the final kill. At the interim kill the difference in adrenal weights between the two groups was not statistically significant (p = .80). However, at the final kill this difference was significant (p = .004). This difference at the final kill can in part be explained by the fact that more benign tumors were present in the exposed animals. For the animals without tumors there is no evidence of a difference between the two groups (p = .13). For the animals with tumors, both exposed and sham-exposed, this difference is still evident (p =.03). The details are given in Table 5.

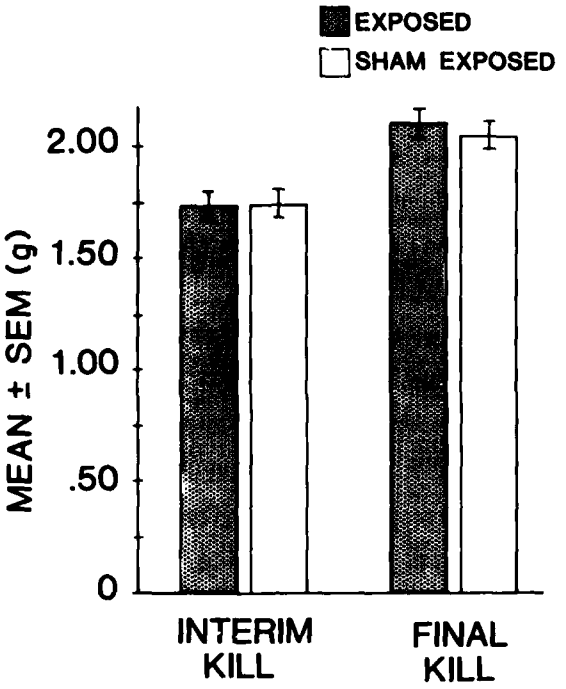

Figure 7. HEART MASS of exposed and sham-exposed animals.

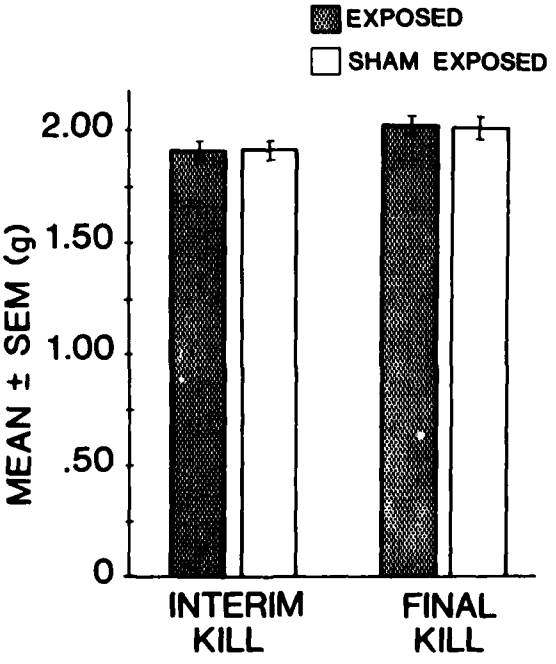

Figure 8. BRAIN MASS of exposed and sham-exposed animals.

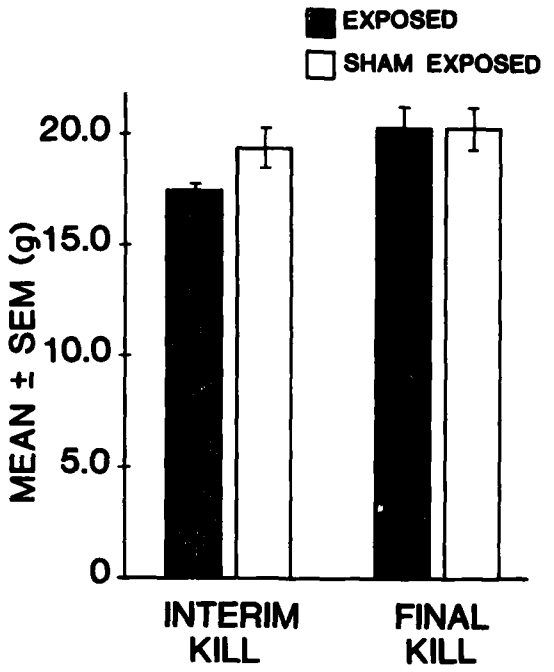

Figure 9. LIVER MASS of exposed and sham-exposed animals.

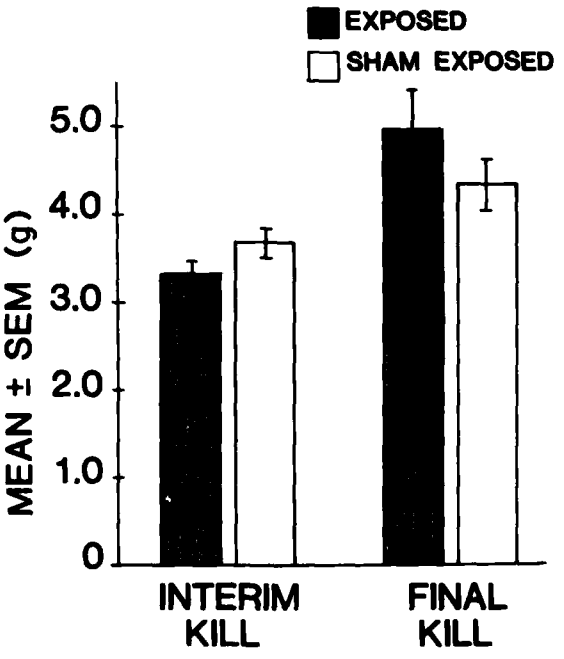

Figure 10. KIDNEY MASS of exposed and sham-exposed animals.

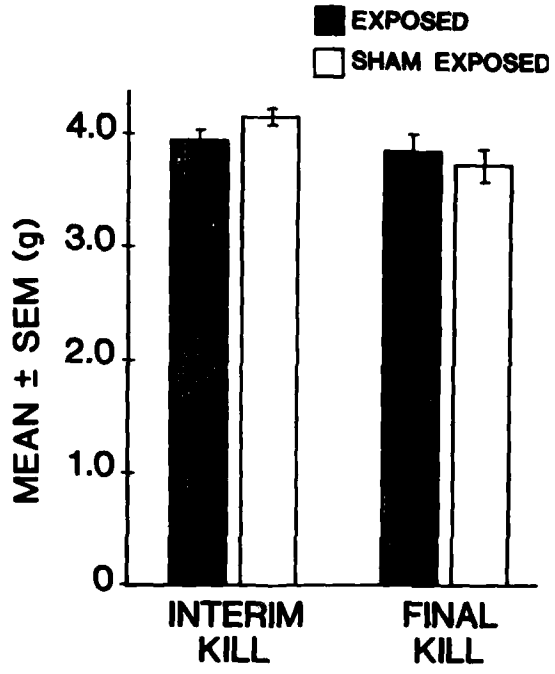

Figure 11. TESTICLE MASS of exposed and sham-exposed animals.

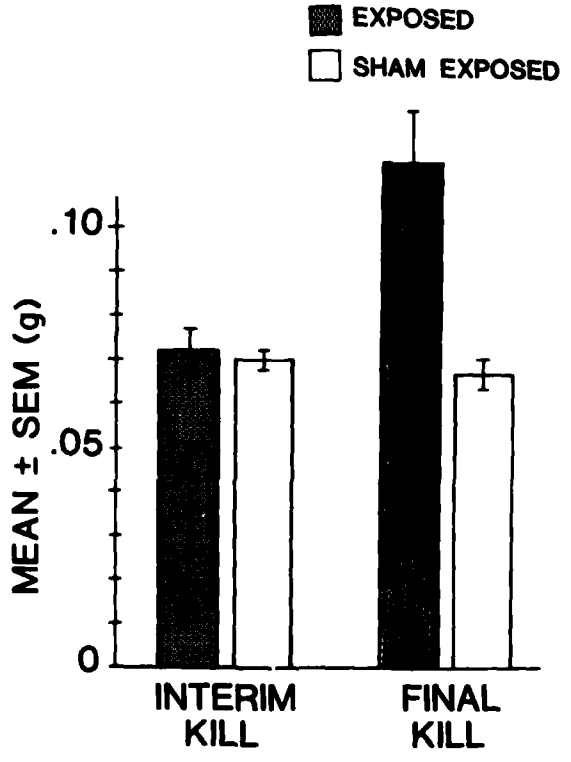

Figure 12. ADRENAL MASS of exposed and sham-exposed animals.

TABLE 5. ANALYSIS OF ORGAN MASS OF EXPOSED AND SHAM-EXPOSED ANIMALS

Descriptive statistics and t-test results

Organ	Exposed M	Exposed SEM	Exposed N	Sham M	Sham SEM	Sham N	t	p	df
INTERIM KILL									
Heart	1.72	0.11	10	1.74	0.07	10	-0.15	.88	18
Brain	1.90	0.04	10	1.91	0.06	10	-0.14	.89	18
Liver	17.44	0.60	10	19.17	1.40	10	-1.12	.28	18
Kidneys	3.35	0.11	10	3.63	0.13	10	-1.57	.13	18
Testicles	3.95	0.08	10	4.17	0.06	10	-2.25	.04	18
Adrenals	0.07	0.007	10	0.07	0.003	10	0.25	.80	18
FINAL KILL									
Heart	2.10	0.09	12	2.06	0.09	11	0.32	.75	21
Brain	2.03	0.03	12	2.02	0.04	11	0.17	.87	21
Liver	20.28	1.06	12	20.25	1.30	11	0.02	.99	21
Kidneys	4.93	0.56	12	4.32	0.28	11	0.97	.36	21
Testicles	3.83	0.16	12	3.73	0.14	11	0.47	.24	21
Adrenal	0.116	0.014	12	0.068	0.004	11	3.23	.004	21
With tumors	0.132	0.016	7	0.069	0.004	4	2.507	.003	9
W/O tumors	0.092	0.017	5	0.068	0.005	7	1.633	.13	10

ANALYSIS OF VARIANCE: Summary of condition, kill, and interaction

	Exposed/Sham F	Exposed/Sham p	Interim/Final F	Interim/Final p	Interaction F	Interaction p
Heart	0.01	.90	13.9	.0006	0.11	.74
Brain	0.00	.98	8.19	.0067	0.05	.83
Liver	0.54	.47	2.94	.09	0.58	.45
Kidneys	0.21	.65	10.13	.003	1.54	.22
Testicles	0.21	.65	5.19	.028	1.63	.21

Carcass Constituents

The eviscerated, decapitated rat carcass was ground in a meat grinder and then, in a commercial blender, homogenized with 3 times its mass of water into a smooth homogenate, free of bone pieces and hair aggregates. Subsamples of this homogenate were drawn and analyzed for the following constituents:

total moisture
total ash
total crude fat
protein-bound nitrogen
nonprotein-bound nitrogen

Moisture, ash, and nitrogen were determined by established analytic procedures (AOAC, 1975). Crude fat was determined by ethyl ether, soxlet extraction. The results were calculated, after correction for the added water, as percentage of total mass.

At the time of the interim kill, 10 exposed and 10 sham-exposed animals were submitted for these evaluations. At the completion of the study, the 23 surviving animals were killed but only 20 carcasses were submitted for carcass-content evaluation.

The mean values for general whole-body composition are presented in Table 6 and Figs. 13 through 16 for the exposed and sham-exposed animals. The two groups are statistically indistinguishable on the basis of either individual *t*-tests for each parameter comparison or an analysis of covariance with body mass as a cofactor.

TABLE 6. ANALYSIS OF CARCASSES OF EXPOSED AND SHAM-EXPOSED ANIMALS

Descriptive statistics and t-test results

		Exposed			Sham					
		M	SEM	N	M	SEM	N	t	p	df
INTERIM KILL										
Body mass	(g)	515.7	11.9	10	534.9	14.1	10	-1.04	.31	18
Moisture	(%)	53.4	1.4	10	52.7	1.6	10	0.32	.75	18
Ash	(%)	8.1	0.5	10	8.7	0.6	10	-0.87	.40	18
Protein N	(%)	7.1	0.3	10	7.3	0.3	10	-0.26	.80	18
Nonprotein N	(%)	0.50	0.02	10	0.53	0.04	10	-0.52	.61	18
Crude fat	(%)	25.6	2.0	10	27.7	1.5	10	-0.84	.41	18
FINAL KILL										
Body mass	(g)	505.1	11.1	10	490.8	21.2	10	0.61	.55	21
Moisture	(%)	58.8	1.7	10	57.6	2.3	10	0.42	.68	18
Ash	(%)	8.6	0.6	10	9.0	0.9	10	-0.37	.72	18
Protein N	(%)	11.5	0.8	10	12.0	0.8	10	-0.47	.64	18
Nonprotein N	(%)	0.58	0.06	10	0.56	0.04	10	0.36	.72	18
Crude fat	(%)	35.5	2.7	10	36.7	3.7	10	-0.27	.79	18

ANALYSIS OF VARIANCE: Summary of condition, kill, and interaction

	Exposed/Sham		Interim/Final		Interaction	
	F	p	F	p	F	p
Body mass	0.03	0.87	3.24	.08	1.21	.28
Moisture	0.28	0.60	8.15	.007	0.02	.88
Ash	0.61	0.44	0.29	.59	0.03	.86
Protein N	0.29	0.59	55.10	.000	0.11	.74
Nonprotein N	0.00	0.97	1.63	.21	0.34	.56
Crude fat	0.41	0.52	13.36	.0008	0.03	.86

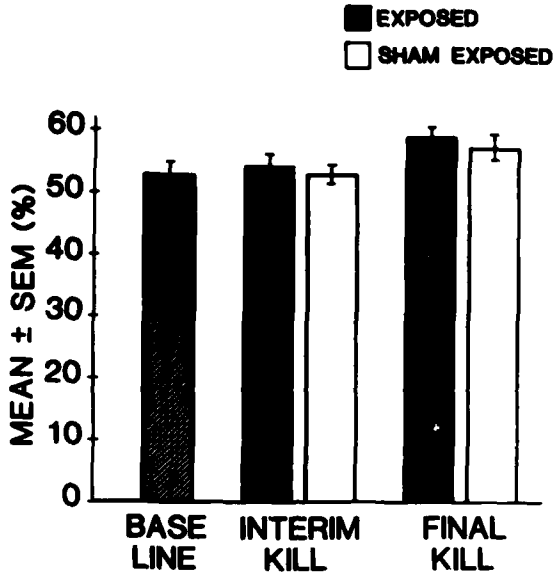

Figure 13. MOISTURE content of carcasses of exposed and sham-exposed animals.

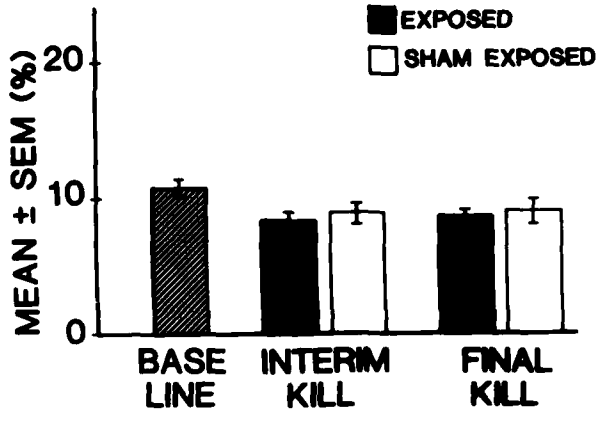

Figure 14. ASH content of carcasses of exposed and sham-exposed animals.

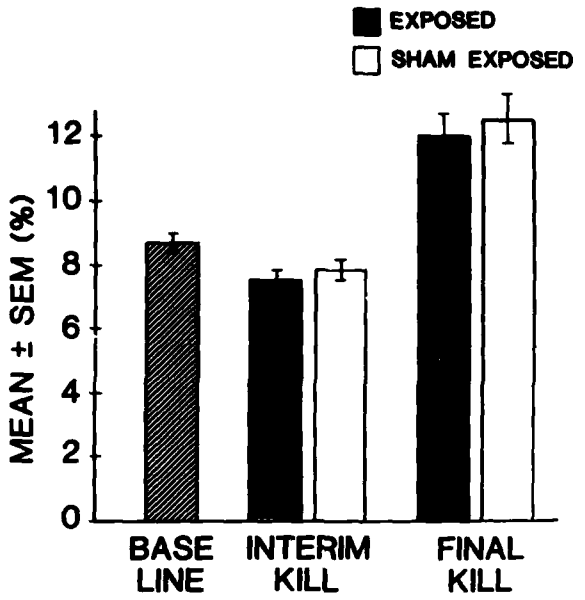

Figure 15. NITROGEN content of carcasses of exposed and sham-exposed animals.

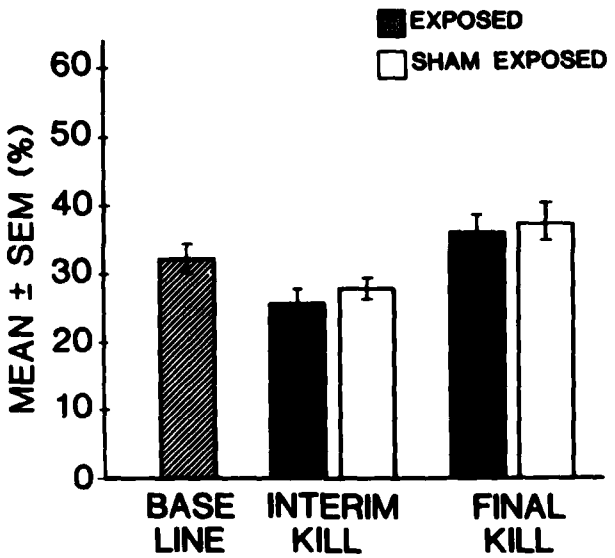

Figure 16. CRUDE-FAT contents of carcasses of exposed and sham-exposed animals.

Fatty Acids

In addition to the general whole-body analysis for the exposed and sham-exposed animals, the crude fat was analyzed with regard to the following fatty acids:

palmitic acid	(C_{16})
palmitoleic acid	(C_{16}-1)
stearic acid	(C_{18})
oleic acid	(C_{18}-1)
linoleic acid	(C_{18}-2)
linolenic acid	(C_{18}-3)

The levels of these fatty acids were determined from the ethyl-ether extract from the crude fat determination. Fatty-acid methyl esters were prepared by refluxing in methanol:sulfuric acid (95:4) for 16 h and were then extracted with petroleum ether and analyzed by gas-liquid chromotography (Metcalfe et al., 1966). The fatty-acid levels were calculated by comparison against commercially obtained standards.

The mean-percentage profiles of these fatty acids are presented in Table 7. None of the pairwise *t*-test comparisons of exposed and sham-exposed animals is significant.

TABLE 7. FATTY ACID CONTENTS OF EXPOSED AND SHAM-EXPOSED ANIMALS

Descriptive statistics and t-test results

	Exposed			Sham					
Organ	M	SEM	N	M	SEM	N	t	p	df
INTERIM KILL									
Palmitic	15.67	0.39	10	15.36	0.41	10	0.52	.61	18
Palmitoleic	4.65	0.27	10	4.88	0.29	10	-0.55	.59	18
Stearic	4.06	0.15	10	3.86	0.19	10	0.77	.45	18
Oleic	29.02	0.25	10	29.22	0.36	10	-0.43	.67	18
Linoleic	41.62	0.45	10	41.56	0.56	10	0.08	.94	18
Linolenic	4.87	0.40	10	5.14	0.52	10	-0.39	.70	18
FINAL KILL									
Palmitic	19.05	0.86	10	18.91	0.61	10	0.13	.90	18
Palmitoleic	5.43	0.50	10	5.54	0.35	10	-0.16	.87	18
Stearic	3.73	0.30	10	3.85	0.24	10	-0.31	.76	18
Oleic	29.42	0.58	10	29.74	0.36	10	-0.45	.66	18
Linoleic	39.57	1.29	10	39.40	0.42	10	0.12	.90	18
Linolenic	2.81	0.46	10	3.06	0.36	10	-0.40	.69	18

Mineral Content

Samples of the homogenate prepared for the fatty acid profile were digested for analysis with nitric and perchloric acids, and the elemental content was determined with a Jarrell Ash, Model 975, ICAP Spectrograph for the following:

aluminum	chromium	phosphate
antimony	cobalt	potassium
arsenic	copper	selenium
barium	iron	silver
beryllium	lead	sodium
bismuth	magnesium	strontium
boron	manganese	tin
cadmium	molybdenum	titanium
calcium	nickel	vanadium

The results of the spectral analysis are presented in Tables 8 and 9 for the interim- and final-kill procedures respectively. The number of viable determinations varies from one test to another, resulting in unequal sample sizes, because the content of some elements was below the sensitivity of the instrumentation. None of the pairwise *t*-test comparisons is statistically significant. Iron content was the only measure on which the exposed and sham-exposed animals appeared to differ, but these differences were not significant.

TABLE 8. SUMMARY OF SPECTROSCOPICALLY DETERMINED MINERAL CONTENTS OF ANIMALS IN THE INTERIM KILL

	N	M	Med.	Mode	SD	Min.	Max.	SE
EXPOSED								
Aluminum	10	27.06	24.50	22.75	12.04	13.0	52.0	3.81
Barium	10	0.97	0.95	0.80	0.25	0.6	1.4	0.10
Boron	3	3.90	5.10	4.40	2.43	1.1	5.5	1.40
Calcium	10	18710.00	17700.00	16912.50	5303.92	12700.0	29550.0	1677.25
Chromium	10	4.93	4.55	4.05	1.48	3.0	7.2	0.47
Copper	8	3.59	3.68	2.50	1.57	1.2	6.4	0.56
Iron	10	167.80	161.50	149.50	46.46	109.0	271.0	14.69
Magnesium	10	581.50	531.00	554.75	111.32	469.0	812.0	35.20
Manganese	10	1.60	1.75	1.90	0.57	0.4	2.4	0.18
Phosphate	10	33550.00	32200.00	29525.00	8370.88	22100.0	51800.0	2647.10
Potassium	10	2963.50	3005.00	2690.00	461.48	2310.0	3830.0	145.93
Selenium	10	0.20	0.19	0.17	0.04	0.1	0.3	0.01
Sodium	10	1301.50	1355.00	1461.25	174.01	1030.0	1605.0	55.02
Strontium	10	4.06	3.84	3.84	1.67	2.5	8.0	0.53
Tin	3	7.13	6.35	4.69	5.72	1.9	13.2	1.81
Titanium	1	2.50	2.50	2.50	0.00	2.5	2.5	0.00
SHAM EXPOSED								
Aluminum	10	52.00	28.15	79.13	77.43	15.5	270.0	24.49
Barium	10	1.09	1.01	1.00	0.43	0.6	2.2	0.14
Boron	4	3.86	4.45	4.59	2.31	0.7	5.9	1.16
Calcium	10	20588.00	17925.00	17647.50	9792.48	9330.0	42600.0	3096.65
Chromium	10	6.01	5.35	4.91	1.81	3.5	9.3	0.57
Copper	9	7.36	4.50	8.20	8.79	1.0	29.8	2.93
Iron	10	256.20	199.50	251.00	174.50	125.0	629.0	55.18
Magnesium	10	591.00	510.00	611.50	201.46	452.0	1090.0	63.71
Manganese	10	2.02	1.75	1.72	1.29	0.8	4.4	0.41
Nickel	1	5.10	5.10	5.10	0.00	5.1	5.1	0.00
Phosphate	10	36310.00	33700.00	31700.00	15116.69	18800.0	70400.0	4780.32
Potassium	10	2724.50	2680.00	2340.00	567.15	1820.0	3900.0	179.35
Selenium	9	0.17	0.16	0.16	0.03	0.1	0.2	0.01
Sodium	10	1254.30	1255.00	1107.00	315.23	836.0	1920.0	99.68
Strontium	10	4.69	3.98	4.01	2.31	2.0	9.9	0.73
Tin	9	9.20	5.95	9.38	8.44	2.6	29.7	2.81
Titanium	2	5.05	5.05	3.03	5.73	1.0	9.1	4.05

TABLE 9. SUMMARY OF SPECTROSCOPICALLY DETERMINED MINERAL CONTENTS OF ANIMALS IN THE FINAL KILL

	N	M	Med.	Mode	SD	Min.	Max.	SE
EXPOSED								
Aluminum	2	65.10	65.10	39.15	73.40	13.2	117.0	51.90
Barium	10	1.83	1.70	1.75	0.65	1.2	3.3	0.21
Boron	9	12.97	11.40	9.30	5.54	5.6	20.4	1.84
Calcium	10	26592.00	24750.00	14590.00	11157.09	4020.0	46300.0	3528.15
Chromium	7	4.89	3.92	5.00	3.40	2.5	12.5	1.29
Copper	10	8.77	7.41	8.29	4.11	5.0	18.2	1.30
Iron	10	153.05	114.50	178.15	105.87	90.2	442.0	33.48
Magnesium	10	681.00	649.50	454.75	193.91	273.0	1000.0	61.32
Manganese	10	2.27	2.34	1.56	0.85	1.0	3.3	0.27
Phosphate	10	52575.00	49125.00	30850.00	19019.95	12800.0	85000.0	6014.64
Potassium	10	2630.50	2707.50	2975.00	500.10	1850.0	3350.0	158.15
Selenium	10	0.29	0.28	0.27	0.05	0.2	0.4	0.02
Sodium	10	1331.40	1325.00	1503.50	298.20	764.0	1750.0	94.30
Strontium	10	6.40	5.97	3.73	2.57	1.2	11.3	0.81
Tin	7	3.67	3.80	3.44	1.46	2.4	6.6	0.55
Vanadium	1	0.67	0.67	0.67	0.00	0.7	0.7	0.00
SHAM EXPOSED								
Aluminum	2	52.25	52.25	39.28	36.70	26.3	78.2	25.93
Barium	10	1.91	1.96	1.38	0.85	0.7	3.5	0.27
Boron	9	15.19	14.90	20.69	7.30	2.9	26.6	2.43
Calcium	10	28116.50	27550.00	20073.75	14449.64	9065.0	53100.0	4569.38
Chromium	9	4.92	4.25	4.57	1.99	3.0	9.3	0.63
Copper	10	10.63	7.42	11.18	9.08	4.2	32.1	2.87
Iron	10	237.78	182.50	207.85	148.80	95.8	544.0	47.05
Magnesium	10	673.10	702.50	535.00	263.17	330.0	1150.0	83.22
Manganese	10	2.31	2.50	1.52	1.35	0.6	4.4	0.43
Phosphate	10	56130.00	53700.00	40875.00	25430.91	22300.0	96600.0	8014.96
Potassium	10	2546.00	2630.00	2132.50	533.15	1640.0	3610.0	168.60
Selenium	10	0.33	0.32	0.29	0.09	0.2	0.5	0.03
Sodium	10	1338.70	1320.00	1162.25	427.53	813.0	2210.0	135.20
Strontium	10	6.77	7.23	9.24	3.21	2.2	11.6	1.02
Tin	6	5.03	2.92	4.28	3.66	2.3	10.2	1.49

Discussion

With one exception, the combined analyses of organ mass, general carcass composition, fatty acid profile, and mineral content provided no evidence that metabolic processes were irreversibly altered in the animals exposed for 13 or 25 months to microwave radiation. A highly significant elevation of adrenal mass was indicated by the 75% increase observed for the exposed rats compared to the sham exposed. However, when the animals with benign tumors in the adrenal gland were separated from those without tumors, the difference became insignificant. For animals with tumors, the adrenal weight was significantly higher in the exposed group than in the sham group. This analysis indicated that the increased adrenal weight was related to the tumors and irrelevant to the metabolic processes in the rats. It was noticed that the mean adrenal mass in the exposed animals without tumors was slightly heavier, but statistically insignificant as compared with those of the sham group. This increase in weight was due to one animal with a hyperplastic adrenal cortex that was secondary to a pituitary tumor.

RESPIRATORY GAS EXCHANGE

The most widely used method of metabolic rate assessment measures respiratory gas exchange and is based on the principle that the chemistry of cellular metabolism requires (consumes) oxygen and produces waste products (e.g., carbon dioxide) in direct proportion to the intensity of the metabolic activity. The sum of all metabolic activity within an organism can be measured as the rate at which oxygen is being removed from the air in the immediate environment of the organism.

One method of respiratory exchange measurement, characterized as an open-circuit system, places an animal in an airtight chamber that has an air intake and an air outlet, with fresh air constantly circulated through the chamber. This allows an animal to remain inside for extended periods of time. As the animal breathes, oxygen is removed from the air within the chamber and the oxygen content of the outgoing air is lower than that of the incoming air. The oxygen concentration in the outlet air fluctuates as a function of the rate at which air flows through the chamber; therefore, the volume of oxygen consumed by an animal must be calculated as the difference between the volumes of oxygen entering and leaving the chamber, expressed as a rate:

$$\dot{V}_{O_2} = F_{I_{O_2}} \dot{V}_I - F_{E_{O_2}} \dot{V}_E \qquad (1)$$

Similarly, carbon dioxide production can be expressed as the difference in the volumes of carbon dioxide entering and leaving the metabolism cage;

$$\dot{V}_{CO_2} = F_{E_{CO_2}} \dot{V}_E - F_{I_{CO_2}} \dot{V}_I \qquad (2)$$

In these and following equations, F_I and F_E denote the fractional concentration of the inspired and expired gases; and $\dot{V}_I$ and $\dot{V}_E$, the respective volumes of the incoming and outgoing air expressed as a rate,

e.g., ml/min. This use of symbols follows that previously established and used throughout the literature (Depocas and Hart, 1957; Otis, 1964; Hill, 1972; Withers, 1977; Fedak et al., 1981).

The above calculations do not require that both $\dot{V}_I$ and $\dot{V}_E$ actually be measured. The incoming volume, $\dot{V}_I$, can be calculated based on measurement of the exhaust volume and the assumption of pulmonary nitrogen equality and the Haldane transformation (Wilmore and Costill, 1973). The assumption of pulmonary nitrogen equality during ventilation asserts that there is no net exchange of nitrogen or other inert gases during respiration. This can be expressed as

$$F_{I_{N_2}} \dot{V}_I = F_{E_{N_2}} \dot{V}_E \tag{3}$$

and rearranged to yield,

$$\dot{V}_I = \frac{F_{E_{N_2}}}{F_{I_{N_2}}} \dot{V}_E \tag{4}$$

Therefore, substitution of equation (4) into equations (1) and (2) allows oxygen consumption and carbon dioxide production to be calculated without measurement of $\dot{V}_I$:

$$\dot{V}_{O_2} = F_{I_{O_2}} \frac{F_{E_{N_2}}}{F_{I_{N_2}}} \dot{V}_E - F_{E_{O_2}} \dot{V}_E \tag{5}$$

$$\dot{V}_{CO_2} = F_{E_{CO_2}} \dot{V}_E - F_{I_{CO_2}} \frac{F_{E_{N_2}}}{F_{I_{N_2}}} \dot{V}_E \tag{6}$$

Since the fractional carbon dioxide concentration in ambient air under normal conditions is essentially zero, i.e. $F_{I_{CO_2}} = 0$, it is generally dropped from calculations (Otis, 1964); and equation (6) can be simplified:

$$\dot{V}_{CO_2} = F_{E_{CO_2}} \dot{V}_E \tag{7}$$

The use of equation (5) to calculate oxygen consumption can be further simplified by eliminating explicit measures of N_2. The sum of all partial pressures of the component gases in ambient air is equal to the total barometric pressure, so the sum of the fractional concentrations of these gases is equal to unity. This relationship holds true for both inlet and outlet air measurements and can be expressed as follows:

$$F_{I_{N_2}} + F_{I_{O_2}} + F_{I_{CO_2}} = 1 \tag{8}$$

$$F_{E_{N_2}} + F_{E_{O_2}} + F_{E_{CO_2}} = 1 \tag{9}$$

These equations can be rearranged to yield

$$F_{I_{N_2}} = 1 - F_{I_{O_2}} - F_{I_{CO_2}} \tag{10}$$

$$F_{E_{N_2}} = 1 - F_{E_{O_2}} - F_{E_{CO_2}} \tag{11}$$

Under normal ambient conditions $F_{I_{CO_2}} = 0$ and $F_{I_{O_2}}$ can be assumed to be a constant equal to 0.2093, then $F_{I_{N_2}} = 0.7907$. $F_{E_{N_2}}$ can then be calculated from the measured values of $F_{E_{O_2}}$ and $F_{E_{CO_2}}$.

The ratio of carbon dioxide production to oxygen consumption is known as the respiratory quotient (RQ),

$$RQ = \frac{\dot{V}_{CO_2}}{\dot{V}_{O_2}} \tag{12}$$

The respiratory quotient is useful as an index of the type of foodstuffs being metabolized by an organism; i.e., proteins, carbohydrates, or fats. The RQ value generally falls within the range between 0.7 (fat) and 1.0 (carbohydrate) (Kleiber, 1961).

Another useful measure of metabolism, suggested by Sacher (1977), is expressed as the ratio of maximum oxygen consumption to minimum oxygen consumption. This measure has been interpreted as an index of activity, health, vigor, and ultimately longevity. We will term this ratio the metabolic quotient (MQ) and define it as follows:

$$MQ = \frac{\max \dot{V}_{O_2}}{\min \dot{V}_{O_2}} \tag{13}$$

The daily measurements of air volume are made under varying ambient conditions of temperature (T_a), barometric pressure (P_B), and water-vapor saturation (ATPS); and prior to use in the above equations, the values obtained must be corrected to dry conditions of standard temperature and pressure (STPD, $0^{o}C$, 760 mmHg) as follows:

$$\dot{V}_{STPD} = \dot{V}_{ATPS} \left(\frac{P_B + P_{H_2O}}{760} \right) \left(\frac{273}{273 + T_a} \right) \tag{14}$$

Method

The cost of acquiring instrumentation capable of simultaneously collecting gas exchange measurements from 200 animals was prohibitive; therefore, only one of the five alcoves in each animal room was selected for this purpose. Within these alcoves, one exposed and one sham-exposed waveguide were outfitted with a modified Plexiglas cage without the usual ventilation holes but with airtight seals, air inlet/outlet ports, and integral waste collectors. These chambers provided an enclosed environment of approximately the same volume (5500 ml) as the standard waveguide cage. The 18 animals within each alcove were placed, two at a time, into these "metabolic cages" instead of their assigned cages for 2-day periods of gas exchange measurement on a rotational basis. As a provision for adaptation to the novel environment, the first-day data from the metabolism cages was not used.

Many technical problems are associated with the use of gas exchange as an index of metabolism. The commercially available oxygen analyzers actually measure the partial pressure of oxygen in the sampled air. The temperature of the air, its relative humidity, the barometric pressure, and the chamber airflow rates all affect such measurement. To simplify the metabolism-monitoring system so that it could be routinely used as part of the daily procedure, we specifically selected the type of equipment used on this project so as to minimize the need to precisely monitor these additional endpoints.

The oxygen analyzer used is a dual-channel unit that has two sample cells for simultaneously measuring the partial pressure of oxygen in two samples of air, expressed as percent oxygen (Applied Electrochemistry, Model S-3A, Sunnyvale, CA). Although both channels sample simultaneously, the data from only one cell at a time can be displayed on the panel meter, with a resolution of 0.01%. Alternatively, the difference between the cells can be displayed with 0.001% resolution. The output voltages from each cell and the cell differential voltage are continuously available from rear panel connectors.

To isolate our measurements from ambient environment changes, we operated the analyzer in the differential mode such that cell 1 was always sampling the ambient air entering the two cages and cell 2 was alternately sampling air exhausted from one of these two cages. Since this analyzer internally references both sample cells to the ambient environment (oxygen concentration, temperature, and pressure) and the cells are calibrated daily under these identical reference conditions, the effects of temperature and humidity changes become negligible. The difference between oxygen in the incoming and exhausted air is being measured directly, so the ambient air conditions again become of minor importance. Operation in the differential mode also eliminates the need to daily calibrate the system with a calibration gas of known absolute oxygen content. Since both cells were internally referenced to ambient air and one cell measured the ambient oxygen level, the daily calibration procedure simply adjusted the instrument to read the accepted ambient value of 20.90%. This value applies only to air samples that have been dried to eliminate the vapor pressure of water (Beaver, 1973), and surge bottles filled with a desiccant ($CaSO_4$, Drierite) removed the water vapor from this system. The Drierite was reconstituted regularly by open drying in a warm oven. A block diagram of the metabolism hardware located in each room is shown in Fig. 17.

Small perturbations in the airflow rates through the system have little effect on the accuracy of the oxygen measurements; however, a very critical aspect is that the calibration procedure be performed under airflow conditions identical to those when actual measurements are taken. The airflow rates chosen for this system were in the range of highest stability for each particular measuring device.

The rate at which air must be drawn through each chamber to sustain a rat was experimentally determined to be approximately 2000-2500 ml/min. This flow rate maintained a .200-.209 concentration of oxygen in the cage so as not to subject the rat to low levels of oxygen. The flow rate also served to restrict the fractional concentration of carbon dioxide to below 0.05 and the temperature rise within the metabolism cage to a level comparable to that found in standard waveguide cages.

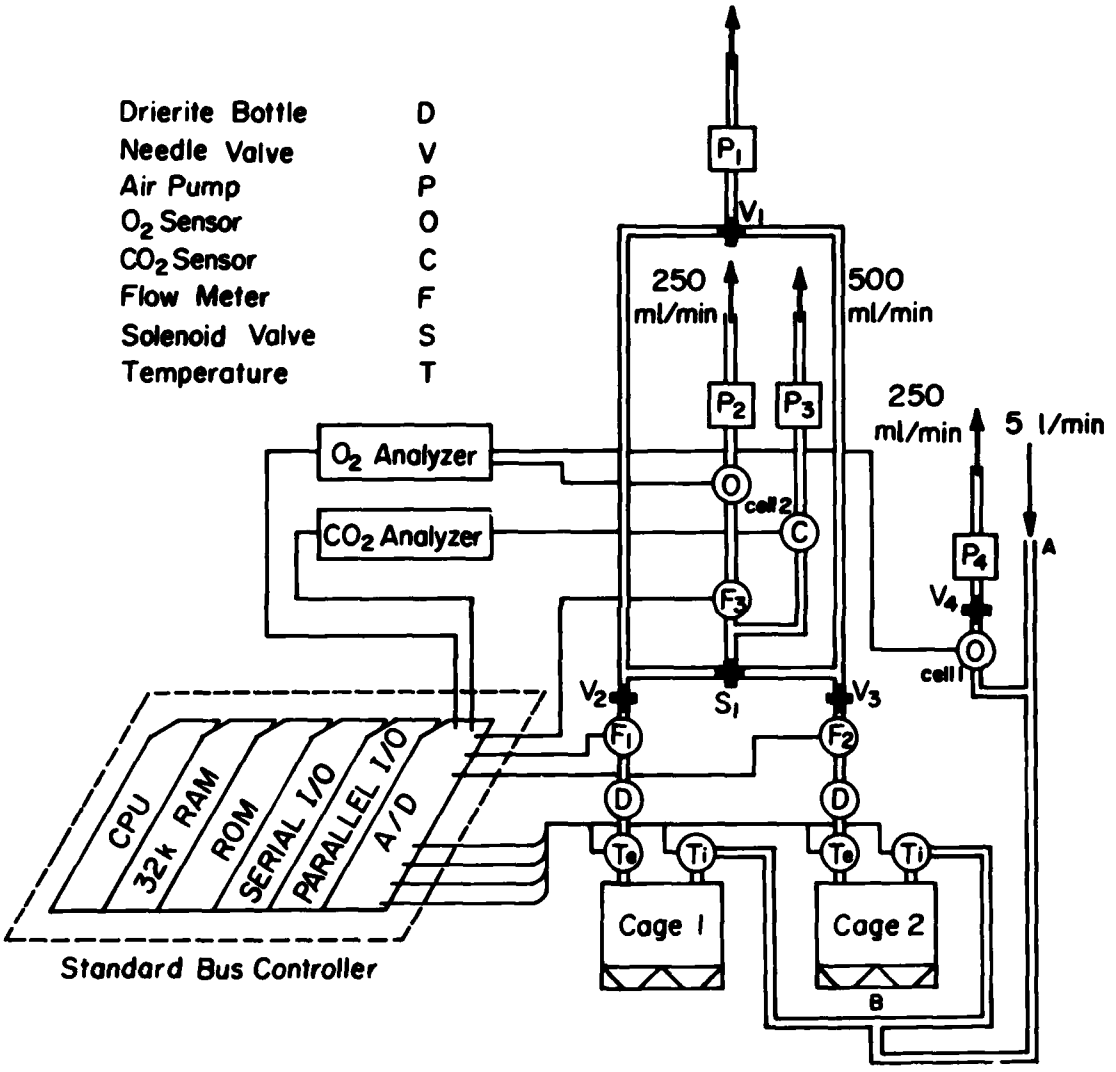

Figure 17. Block diagram of apparatus for measuring respiratory gas.

The constant flow of ambient air was continually drawn through the chambers by the master pump, P_1. By adjusting valve V_1, located directly on the pump, we set the master pump to draw air at approximately 4000-5000 ml/min. This flow was not monitored directly, only as the sum of the individual airflows drawn through each chamber. The balance of flow between the two chambers was controlled by flow valves V_2 and V_3. The mass flow through each chamber was continuously measured by flow meters F_1 and F_2 (Kurz Instruments, Model #565-5, Carmel Valley, CA). These thermal mass-flow sensors are temperature and pressure compensated and provide a linear output for direct reading of mass flow (0.0-5.0 l/min $\pm$ 2%). The sensors were calibrated periodically using an NBS-traceable (National Bureau of Standards) flow calibrator (Kurz Instruments, Model #540). The air flowing into the chambers was derived from a common inlet at point A and equally divided at point B.

The computer built into the metabolism control unit electrically switched a solenoid-actuated flow valve, S_1, so that the air sampled for cell #2 was alternately drawn from cage #1 and cage #2 at 30-s intervals. All interconnecting tubing for the two chambers were of equal length to avoid pressure changes within the system when switching from one chamber to the other. During the first 30-s interval each minute, the computer logged the data for cage #1 once each second. During the second 30-s interval, the data from cage #2 was collected. The data collected from the first 20 s of each 30-s sampling period was discarded by the metabolic control computer since this length of time was required for complete washout of the air lines contaminated with gases from the cage sampled during the previous 30-s period. The averages of the 10 remaining samples in each 30-s period were averaged and retained. A small volume sample of the exhaust air was withdrawn and passed through the sensor of a Beckman LB-2 Carbon Dioxide Analyzer (Beckman Instruments, Schiller Park, IL). This analyzer was calibrated regularly using bottled carbon dioxide gas of known concentration. Carbon dioxide readings were made with an accuracy of 0.1%. The air temperature entering and leaving each chamber was measured by a temperature-sensitive solid-state sensor. Signal conditioning and A/D conversion circuitry provided a direct digital readout with .1^{o}C resolution.

The minute-by-minute averages for both cages for the entire daily monitoring period were stored in the control computer and transferred each morning to the main room computer. The data included:

fractional differential oxygen concentration ($F_{I_{O_2}} - F_{E_{O_2}}$)

fractional exhaust carbon dioxide concentration ($F_{E_{CO_2}}$)

airflow rates through flow meters 1 and 2 ($\dot{V}_{ATPD}$)

airflow rate through flow meter 3 as a check for proper operation of the air-sampling pump

cage air temperature rise ($T_{outlet} - T_{inlet}$)

The daily sampling period generally extended from approximately 1200 to 0800 the following morning. However, since the actual start/stop time on any particular day was a function of other activities within the animal facility, the sampling period covered by the analysis was standardized to a shorter (17-h) period, from 1300 daily to 0600. The data collected at 1-min increments during this period are reblocked for analysis in 60-min periods.

Two sets of respiratory measurements are presented in the following sections. The first set was collected from surviving animals in the two metabolism alcoves during months 15 through 22 of the 25-month low-level exposure project. The animals were approximately 17-24 months of age during this period, with an average weight of 550-600 g, and will be referred to as the "mature" animals.

The decision to measure respiratory gas was made late in the planning of this project, and delays obtaining the required apparatus and assembling a working system prevented data collection from the same animals during early phases of the exposure period. Therefore, after the terminal kill of the mature animals, 36 additional SPF animals were obtained from the same supplier and housed in two alcoves under the same conditions as maintained

during the long-term project. Half of the animals were housed in exposure waveguides; half in sham exposure. The same daily caretaking procedures, as outlined in Volume 1 of this series of reports, were followed by the identical personnel. After animal adaptation, microwave radiation was administered for 3 months, during which time respiratory gas exchange was measured daily. The animals were 2-5 months old during this period of metabolic data collection and weighed between 300 and 400 g. These animals are referred to as the "young" animals during subsequent discussion.

The rotational schedule of placement within the gas-exchange measurement cages allowed each mature animal four to ten 2-day periods of measurement, or "rounds," dependent upon its longevity in the study. After data removal due to equipment/technician error, 48 seventeen-hour data samples were available for analysis from the exposed mature animals and 61 from the sham-exposed animals. The young animals produced two rounds of data each, resulting in 56 total data samples from the exposed and sham-exposed animals.

Two sets of analyses were completed on these data. The first set viewed all 17-h samples as independent, ignoring the fact that an animal contributed not just one sample but one from each round during which it survived. This independence assumption is questionable but does allow utilization of all available data. The second set viewed the samples from each round separately, with each animal contributing only one sample per round. Conclusions from the two sets of analyses are similar. In the following section, the first set of analyses will be presented completely, then the second set.

Results

A typical data profile collected from an animal housed in the metabolic apparatus is presented in Fig. 18. The data covers approximately 11 h, from 2000 to 0700. The lower bar graph in the figure presents the difference in air temperature between the air entering and leaving the chamber. The upper bar graphs show the rates of oxygen consumption and carbon dioxide production.

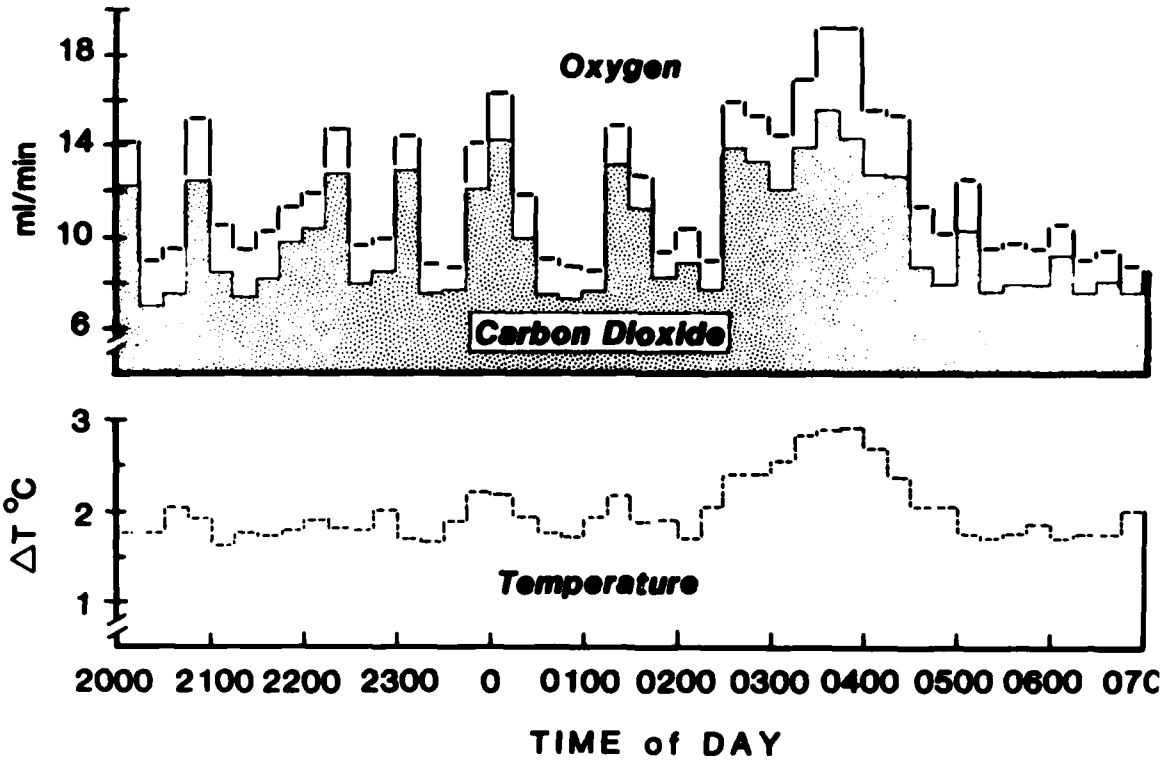

Figure 18. Example of data collected from an animal during an 11-h period (oxygen consumption, carbon dioxide production, and cage temperature fluctuations).

Animals have circadian rhythms in food intake, body temperature, and metabolic rate; so the data collection period was divided into two periods prior to analysis. The diurnal period was 1300-1900 (room lighting was turned off at 1900 daily); the nocturnal period, 1900-0600. The diurnal period consisted of six 1-h periods; the nocturnal period, eleven 1-h periods. Summary statistics for the first analysis for mature and young animals are presented in Tables 10 through 19. These tables include mean, standard error, sample size, and the univariate t-test statistic for each hourly comparison between exposed and sham-exposed treatment groups.

The correlation matrices for each of the variables revealed highly correlated hour-to-hour observations. The univariate t-statistic was not considered sufficient to evaluate the results because it provides no protection against the effect of positive correlation among the subsets nor the possibility that individual tests may be significant due to chance; therefore, the multivariate approach was also used, employing the Hotelling T^2 statistic. Based on the bivariate plots of the data, the assumption of multivariate normality seemed tenable for these data. For some of the variables being considered, the assumption of equal covariance matrices was dubious. In the case of the younger animals, however, the sample sizes were equal, and the unequal covariance matrices therefore have little or no effect upon the size of the type I error or the power function. In the case of the mature animals, the sample sizes were larger and were therefore covered by the multivariate central limit theorem (Ito and Schull, 1964).

Statistical analyses were performed on both the raw oxygen consumption and carbon dioxide production rates (ml/min per rat) and the weighted rates (ml/min per kg). In all instances similar results were obtained, so only the weighted data will be presented in the following section.

Average hourly oxygen consumption for the mature animals is presented in Fig. 19 and Table 10. Although the exposed animals exhibit a slight but consistent elevation of oxygen consumption, none of the individual hourly pairwise t-test comparisons indicates a significant difference between treatment conditions during either the diurnal or nocturnal portions of the 17-h cycle. The multivariate Hotelling T^2 statistic for neither the diurnal period (F = 1.0, p = .37, df = 6,102) nor the nocturnal period (F = .37, p = .92, df = 11,97) indicates any significant overall difference.

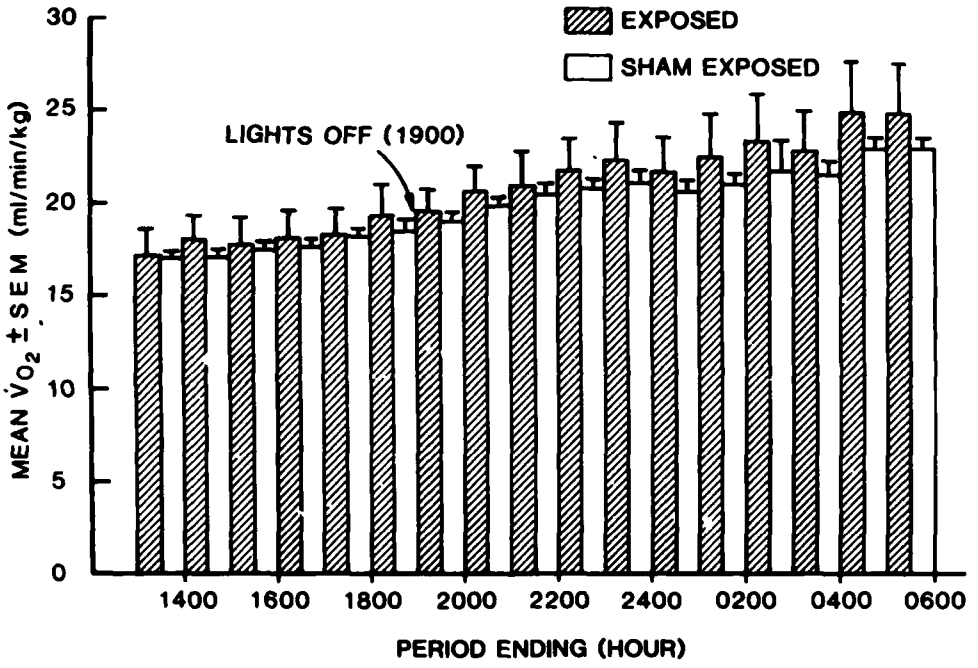

Figure 19. Average hourly oxygen consumption for mature exposed and sham-exposed rats throughout 17-h analysis period.

TABLE 10. SUMMARY OF AVERAGE HOURLY OXYGEN CONSUMPTION FOR MATURE RATS (ml/min per kg)

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	17.3	1.30	48	17.0	0.45	61	0.24	.81	107
2	18.1	1.32	48	17.1	0.44	61	0.78	.44	107
3	17.8	1.46	48	17.5	0.49	61	0.26	.80	107
4	18.2	1.39	48	17.6	0.51	61	0.42	.68	107
5	18.4	1.34	48	18.2	0.52	61	0.16	.87	107
6	19.4	1.57	48	18.5	0.56	51	0.59	.56	107
	$T^2 = 6.96$ $F = 1.01$ $p = .37$ df = 6,102								
7	19.6	1.25	48	19.0	0.53	61	0.49	.63	107
8	20.6	1.44	48	19.8	0.56	61	0.60	.56	107
9	20.9	1.74	48	20.4	0.60	61	0.29	.77	107
10	21.7	1.68	48	20.7	0.62	61	0.62	.54	107
11	22.3	1.94	48	21.0	0.61	61	0.74	.46	107
12	21.6	1.85	48	21.0	0.59	61	0.64	.52	107
13	22.4	2.38	48	20.9	0.63	61	0.70	.49	107
14	23.1	3.00	48	21.6	0.65	61	0.65	.52	107
15	22.7	2.22	48	21.4	0.70	61	0.59	.56	107
16	24.8	2.61	48	22.7	0.67	61	0.85	.40	107
17	24.1	2.63	48	22.7	0.70	61	0.81	.42	107
	$T^2 = 4.44$ $F = 0.37$ $p = .92$ df = 11,97								

Average hourly carbon dioxide production is presented in Fig. 20 and Table 11 for the mature animals. Again, none of the pairwise t-test comparisons is significant. The Hotelling T^2 statistics for the diurnal period (F = 1.01, p = .43, df = 6,102) and the nocturnal period (F = 0.25, p = .99, df = 11,97) also indicate no overall differences between treatment conditions.

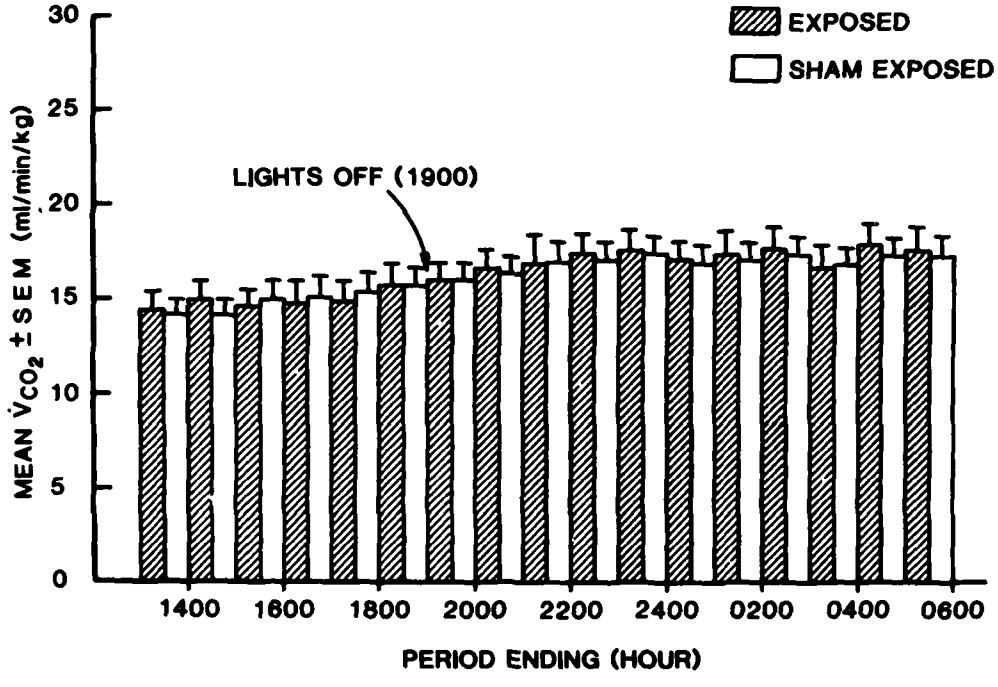

Figure 20. Average hourly carbon dioxide production for mature exposed and sham-exposed rats throughout 17-h analysis period.

TABLE 11. SUMMARY OF AVERAGE HOURLY CARBON DIOXIDE PRODUCTION FOR MATURE RATS (ml/min per kg)

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	14.4	1.18	48	14.2	0.32	61	0.23	.82	107
2	15.0	1.12	48	14.2	0.31	61	0.69	.50	107
3	14.6	1.14	48	15.0	0.77	61	-0.31	.76	107
4	14.8	1.07	48	15.1	1.06	61	-0.18	.86	107
5	14.9	1.06	48	15.4	1.09	61	-0.32	.74	107
6	15.8	1.31	48	15.7	1.07	61	0.06	.95	107
	T^2 = 6.36			F = 1.01			p = .43	df = 6,102	
7	16.0	1.10	48	16.0	1.08	61	0.02	.99	107
8	16.7	1.17	48	16.4	1.04	61	0.19	.85	107
9	17.0	1.41	48	17.0	1.10	61	-0.06	.96	107
10	17.4	1.40	48	17.0	1.10	61	0.19	.84	107
11	17.7	1.43	48	19.3	1.14	61	0.20	.84	107
12	17.1	1.22	48	16.9	1.11	61	0.10	.92	107
13	17.4	1.58	48	17.0	1.08	61	0.23	.81	107
14	17.6	1.68	48	17.3	1.12	61	0.17	.86	107
15	16.8	1.50	48	16.8	1.14	61	-0.03	.97	107
16	17.8	1.54	48	17.3	1.11	61	0.22	.82	107
17	17.6	1.60	48	17.3	1.14	61	0.19	.85	107
	T^2 = 3.09			F = 0.25			p = .99	df = 11,97	

The average hourly respiratory quotient for the mature animals is presented in Fig. 21 and in Table 12, using a discontinuous scale that brackets the accepted range of RQ values. None of the hourly t-test comparisons indicates a significant difference between exposed and sham-exposed animals. This is supported by the T^2 statistic for the diurnal (F = 0.32, p = .93, df = 6,102) and nocturnal (F = 0.25, p = .99, df = 11,97) portions of the cycle.

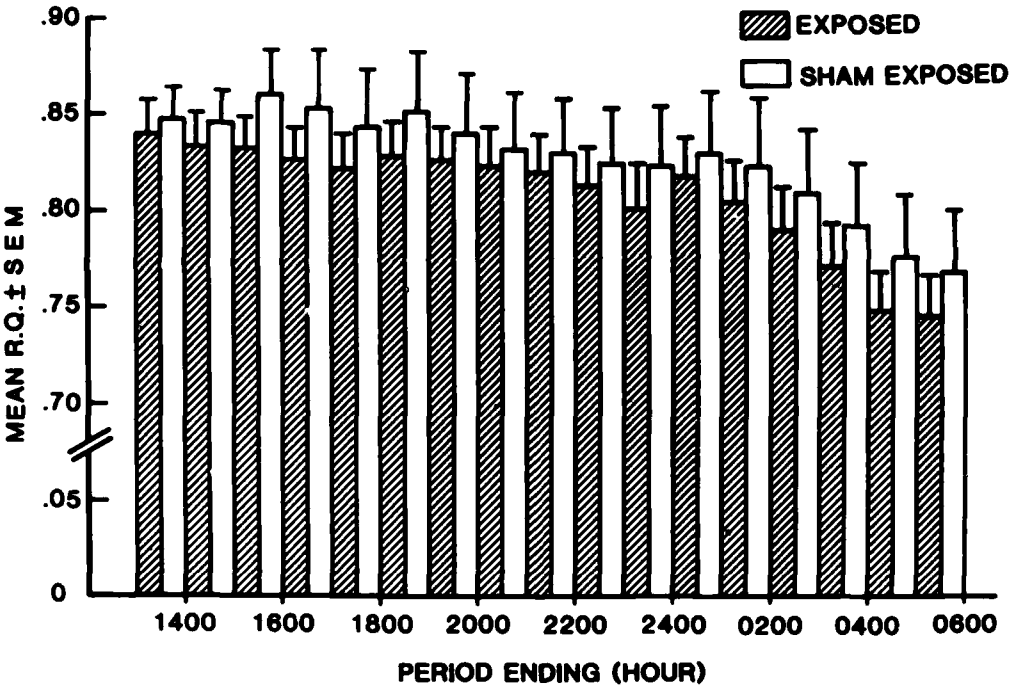

Figure 21. Average hourly respiratory quotient (RQ) for mature rats (RQ = carbon dioxide production/oxygen consumption). The discontinuous scale brackets the accepted RQ range.

TABLE 12. SUMMARY OF AVERAGE HOURLY RESPIRATORY QUOTIENT FOR MATURE RATS

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	0.84	0.02	48	0.85	0.02	61	-0.32	.75	107
2	0.83	0.02	48	0.84	0.02	61	-0.42	.64	107
3	0.83	0.02	48	0.87	0.02	61	-0.90	.37	107
4	0.83	0.02	48	0.85	0.03	61	-0.71	.48	107
5	0.82	0.02	48	0.84	0.03	61	-0.55	.58	107
6	0.83	0.02	48	0.85	0.03	61	-0.58	.56	107
	$T^2 = 2.00$ $F = 0.32$ $p = .93$ df = 6,102								
7	0.83	0.02	48	0.84	0.03	61	-0.36	.72	107
8	0.82	0.02	48	0.83	0.03	61	-0.22	.82	107
9	0.82	0.02	48	0.83	0.03	61	-0.25	.82	107
10	0.81	0.01	48	0.82	0.02	61	-0.28	.78	107
11	0.81	0.02	48	0.82	0.03	61	-0.47	.64	107
12	0.82	0.02	48	0.83	0.03	61	-0.30	.76	107
13	0.81	0.02	48	0.82	0.03	61	-0.42	.67	107
14	0.79	0.02	48	0.81	0.03	61	-0.43	.67	107
15	0.78	0.02	48	0.79	0.03	61	-0.52	.60	107
16	0.75	0.02	48	0.78	0.03	61	-0.65	.51	107
17	0.75	0.02	48	0.77	0.03	61	-0.55	.58	107
	$T^2 = 3.08$ $F = 0.25$ $p = .99$ df = 11,97								

The ratio of the average hourly maximum/minimum oxygen consumption (MQ) is presented in Fig. 22 and Table 13. Only one of the individual hourly t-test comparisons during the daytime indicates a significant difference between treatment conditions (h 3, t = -3.01, p = .003, df = 107). None of the pairwise comparisons during the nighttime portion of the cycle indicates any significant difference between groups. The Hotelling T^2 statistics for the daytime (F = 1.77, p = .11, df = 6,102) and the nighttime (F = 1.20, p = .30, df = 11,97) indicate no overall differences between groups.

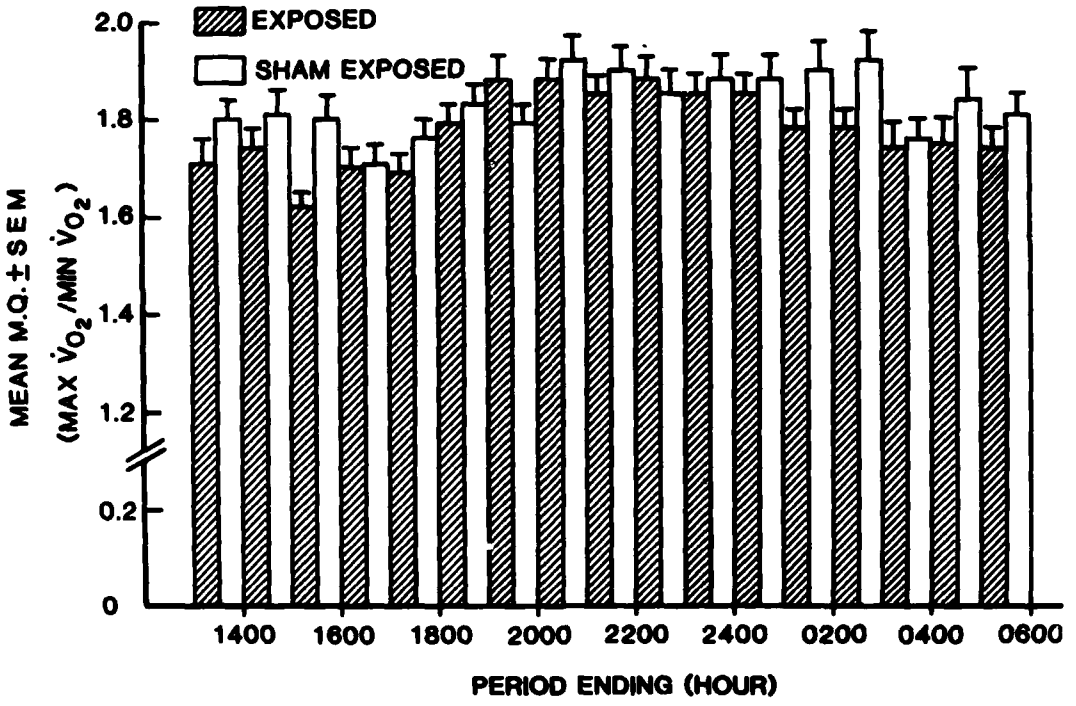

Figure 22. Average hourly metabolism quotient (MQ) for mature rats (MQ = maximum/minimum oxygen consumption). The discontinuous scale emphasizes minor differences between the treatment conditions.

TABLE 13. SUMMARY OF AVERAGE HOURLY METABOLISM QUOTIENT FOR MATURE RATS

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	1.71	0.04	48	1.80	0.05	61	-1.30	.20	107
2	2.73	0.04	48	1.81	0.05	61	-1.22	.27	107
3	1.62	0.04	48	1.80	0.04	61	-3.01 *	.003	107
4	1.70	0.03	48	1.71	0.04	61	-0.10	.92	107
5	1.70	0.04	48	1.76	0.05	61	-1.13	.26	107
6	1.80	0.04	48	1.82	0.04	61	-0.64	.52	107
	T^2 = 11.17			F = 1.77			p = .11	df = 6,102	
7	1.89	0.04	48	1.80	0.04	61	1.47	.14	107
8	1.88	0.04	48	1.92	0.04	61	-0.66	.51	107
9	1.85	0.04	48	1.90	0.06	61	-0.64	.53	107
10	1.88	0.47	48	1.85	0.04	61	0.46	.65	107
11	1.84	0.04	48	1.89	0.04	61	-0.62	.54	107
12	1.85	0.04	48	1.88	0.05	61	-0.39	.70	107
13	1.78	0.05	48	1.90	0.05	61	-1.79	.08	107
14	1.77	0.04	48	1.02	0.07	61	-1.61	.11	107
15	1.74	0.05	48	1.77	0.04	61	-0.40	.69	107
16	1.75	0.03	48	1.84	0.06	61	-1.13	.26	107
17	1.74	0.04	48	1.81	0.04	61	-0.93	.33	107
	T^2 = 14.53			F = 1.20			p = .30	df = 11,97	

*Statistically significant.

The average hourly cage temperature rise is presented for the mature animals in Fig. 23, using a discontinuous scale that exaggerates small variations in temperature rise, and in Table 14. During the diurnal period the individual pairwise t-test comparisons and the Hotelling T^2 statistic ($F = 3.25$, $p = .006$, df = 6,102) indicate that the rise in cage temperature is significantly higher (approximately .1-.2° C) for the exposed animals than for the sham exposed. This difference diminishes during the nighttime, becoming nonsignificant during the early morning hours. However, the T^2 statistic indicates an overall significant difference between treatment conditions during the nocturnal period ($F = 1.94$, $p = .04$, df = 11,97).

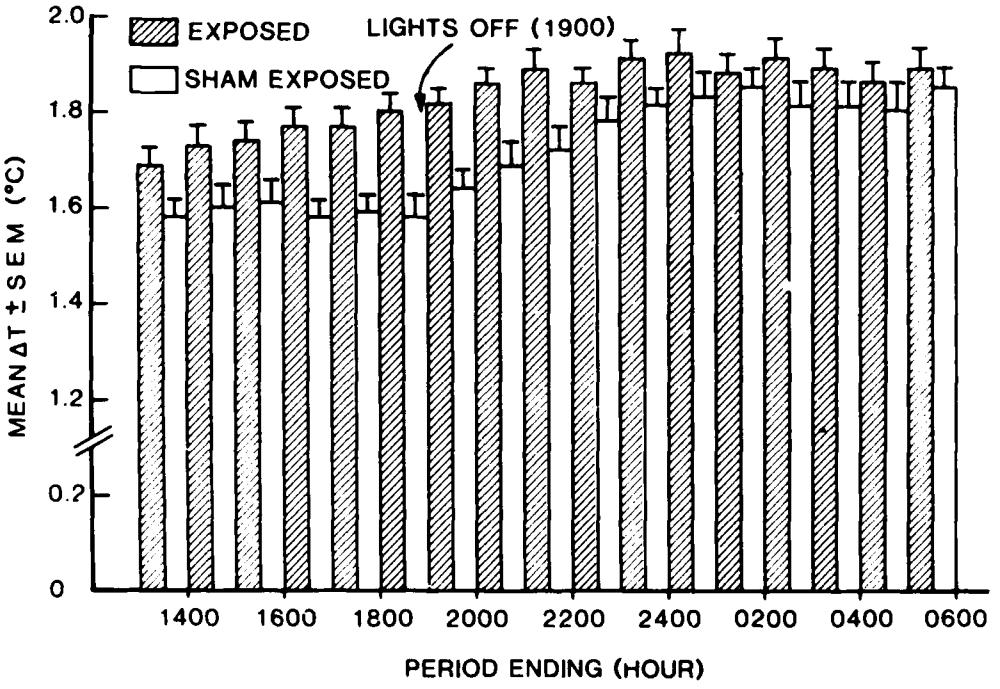

Figure 23. Average hourly cage temperature rise for mature rats ($T_{delta} = T_{exhaust} - T_{intake}$). The discontinuous scale emphasizes minor differences between the treatment conditions.

TABLE 14. SUMMARY OF AVERAGE HOURLY CAGE TEMPERATURE RISE FOR MATURE RATS (^{o}C)

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	1.69	0.03	48	1.57	0.04	61	1.86	.07	107
2	1.73	0.04	48	1.60	0.05	61	2.12 *	.04	107
3	1.74	0.04	48	1.61	0.05	61	2.09 *	.04	107
4	1.77	0.04	48	1.58	0.04	61	3.13 *	.002	107
5	1.77	0.04	48	1.59	0.04	61	3.12 *	.002	107
6	1.80	0.04	48	1.58	0.05	61	3.52 *	.001	107
	$T^2 = 20.48$			$F = 3.25$			$p = .006$	df = 6,102	
7	1.81	0.03	48	1.64	0.04	61	3.31 *	.001	107
8	1.86	0.03	48	1.69	0.04	61	2.92 *	.004	107
9	1.89	0.04	48	1.72	0.04	61	2.68 *	.01	107
10	1.86	0.03	48	1.78	0.04	61	1.46	.14	107
11	1.91	0.04	48	1.81	0.04	61	1.66	.10	107
12	1.92	0.04	48	1.83	0.04	61	1.45	.15	107
13	1.88	0.04	48	1.84	0.04	61	0.54	.59	107
14	1.90	0.04	48	1.80	0.04	61	1.56	.12	107
15	1.89	0.04	48	1.81	0.05	61	1.23	.22	107
16	1.86	0.04	48	1.81	0.05	61	0.89	.37	107
17	1.89	0.04	48	1.85	0.04	61	0.72	.47	107
	$T^2 = 23.6$			$F = 1.94$			$p = .04$	df = 11,97	

*Statistically significant.

Average hourly oxygen consumption for the young animals is presented in Fig. 24 and Table 15. There appears to be a pronounced rise in oxygen consumption coincident with the start of the lights-off condition at 1900 and a general elevation of oxygen consumption during the nighttime hours. None of the individual t-test comparisons indicates a significant difference between treatment conditions during either portion of the daily cycle. The multivariate T^2 statistic for the diurnal period (F = 1.67, p = .15, df = 6,49) indicates no significant difference between groups. However, the T^2 statistic for the nocturnal period does indicate a significant difference between the treatment conditions (F = 2.29, p = .025, df = 11,44).

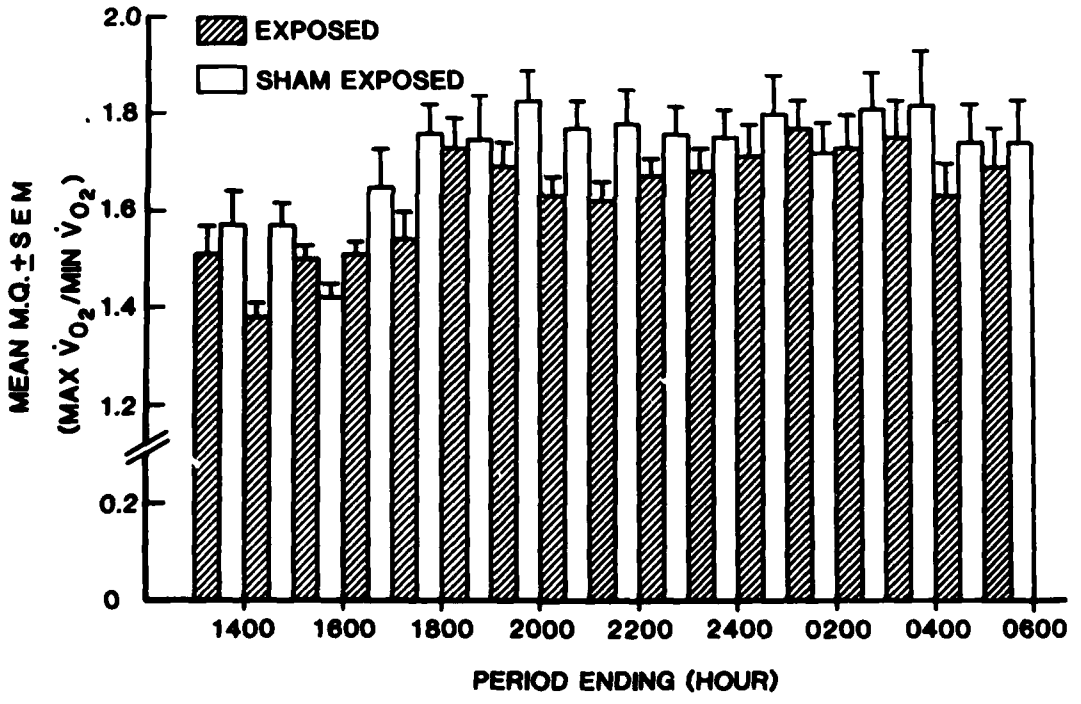

Figure 24. Average hourly oxygen consumption for young rats throughout 17-h analysis period.

TABLE 15. SUMMARY OF AVERAGE HOURLY OXYGEN CONSUMPTION FOR YOUNG RATS (ml/min per kg)

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	20.9	0.96	28	20.1	0.94	28	0.06	.96	54
2	21.7	1.36	28	22.0	1.27	28	-0.13	.90	54
3	23.1	1.43	28	23.3	1.42	28	-0.07	.84	54
4	25.2	1.60	28	24.8	1.52	28	0.21	.83	54
5	24.8	1.30	28	26.1	1.32	28	-0.70	.49	54
6	26.5	1.26	28	26.5	1.26	28	-0.03	.98	54
	$T^2 = 11.04$ $F = 1.67$ $p = .15$ df = 6,49								
7	29.2	1.02	28	30.2	1.10	28	-0.67	.50	54
8	29.2	1.01	28	30.8	1.05	28	-0.92	.37	54
9	29.3	1.13	28	29.5	1.33	28	-0.14	.89	54
10	29.3	1.17	28	29.4	1.19	28	-0.09	.92	54
11	29.1	1.21	28	31.0	1.22	28	-1.11	.27	54
12	29.3	1.36	28	30.8	1.37	28	-0.81	.42	54
13	29.0	1.16	28	30.0	1.50	28	-0.54	.59	54
14	28.9	1.42	28	30.0	1.24	28	-0.58	.56	54
15	29.9	1.54	28	29.5	1.41	28	0.20	.84	54
16	28.9	1.41	28	29.6	1.37	28	-0.34	.73	54
17	28.6	1.54	28	29.8	1.37	28	-0.60	.54	54
	$T^2 = 30.98$ $F = 2.29$ $p = .025$ df = 11,44 *								

*Statistically significant.

Average hourly carbon dioxide production is presented in Fig. 25 and Table 16 for the young animals. Carbon dioxide production shows a marked rise coincident with the lights-off condition and an overall elevation during the nocturnal period compared to the diurnal. None of the t-test comparisons during the daytime indicates a significant difference between groups. Although the minor differences between groups apparent on an hourly basis during the daytime show no consistent pattern, the Hotelling T^2 statistic is significant (F = 2.73, p = .023, df = 6,49). During the nighttime hours, only two hourly t-tests are significant and there is one reversal in the apparent tendency for the exposed animals to have lower rates of carbon dioxide production. The Hotelling T^2 statistic, however,indicates a significant overall difference between treatment conditions during this period (F = 2.91, p = .006, df = 11,44).

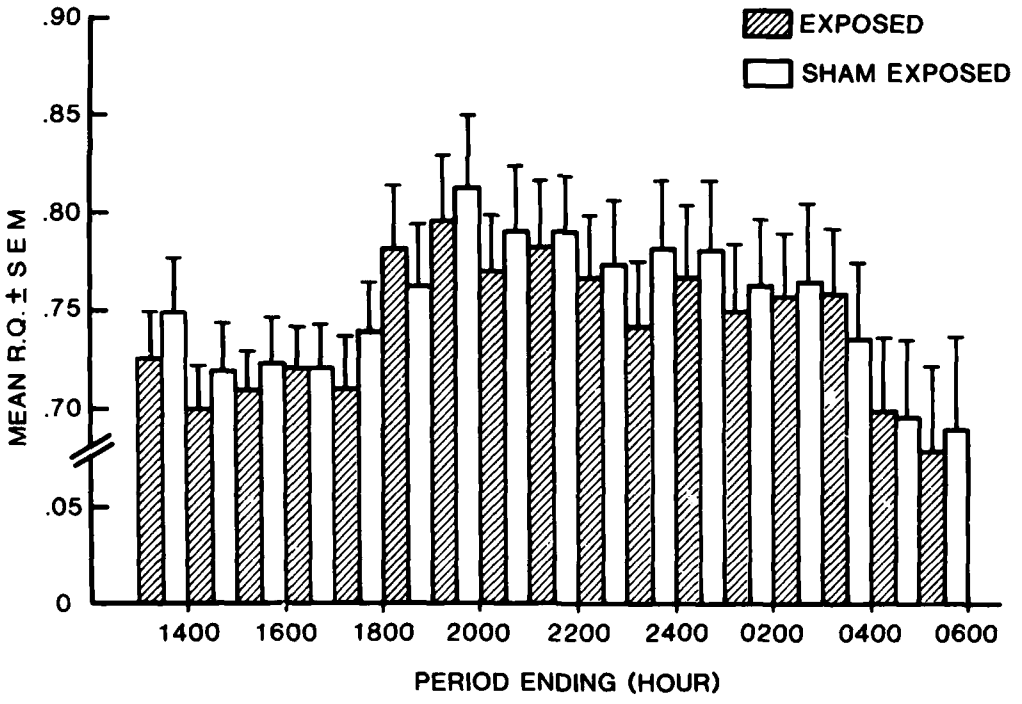

Figure 25. Average hourly carbon dioxide production for young rats throughout 17-h analysis period.

TABLE 16. SUMMARY OF AVERAGE HOURLY CARBON DIOXIDE PRODUCTION FOR YOUNG RATS (ml/min per kg)

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	15.8	0.52	28	16.3	0.50	28	-0.68	.50	54
2	15.8	0.71	28	16.3	0.62	28	-0.62	.54	54
3	17.3	0.95	28	17.6	0.84	28	-0.20	.84	54
4	19.2	1.16	28	18.8	1.05	28	0.25	.80	54
5	18.3	0.71	28	20.3	0.87	28	-1.80	.07	54
6	21.4	0.67	28	21.0	0.73	28	0.40	.67	54
	T^2 = 18.04 F = 2.73 p = .023 df = 6,49 *								
7	24.3	0.76	28	25.5	0.58	28	-1.24	.21	54
8	23.6	0.60	28	25.5	0.64	28	-2.16 *	.04	54
9	23.8	0.59	28	24.2	0.62	28	-0.46	.65	54
10	23.3	0.68	28	23.6	0.65	28	-0.35	.73	54
11	22.4	0.56	28	25.2	0.73	28	-3.02 *	.004	54
12	23.1	0.73	28	24.7	0.59	28	-1.71	.09	54
13	22.4	0.55	28	23.5	0.81	28	-1.09	.28	54
14	22.6	0.63	28	23.7	0.80	28	-1.09	.28	54
15	23.4	0.96	28	22.2	0.86	28	0.94	.35	54
16	20.6	0.50	28	21.2	0.60	28	-0.73	.46	54
17	20.2	0.94	28	21.6	1.01	28	-0.96	.33	54
	T^2 = 39.26 F = 2.91 p = .006 df = 11,44 *								

*Statistically significant.

The average hourly respiratory quotients of the young animals are presented in Fig. 26 and Table 17. Again, the RQ values appear to have a generally higher level during the nocturnal period than the diurnal. None of the daytime t-test comparisons indicates a significant difference between treatment groups, and the T^2 statistic supports this finding (F = 2.00, p = .08, df = 6,49). During the nighttime hours, none of the t-test comparisons is significant and the T^2 statistic indicates no overall difference (F = 1.76, p = .09, df = 11,44).

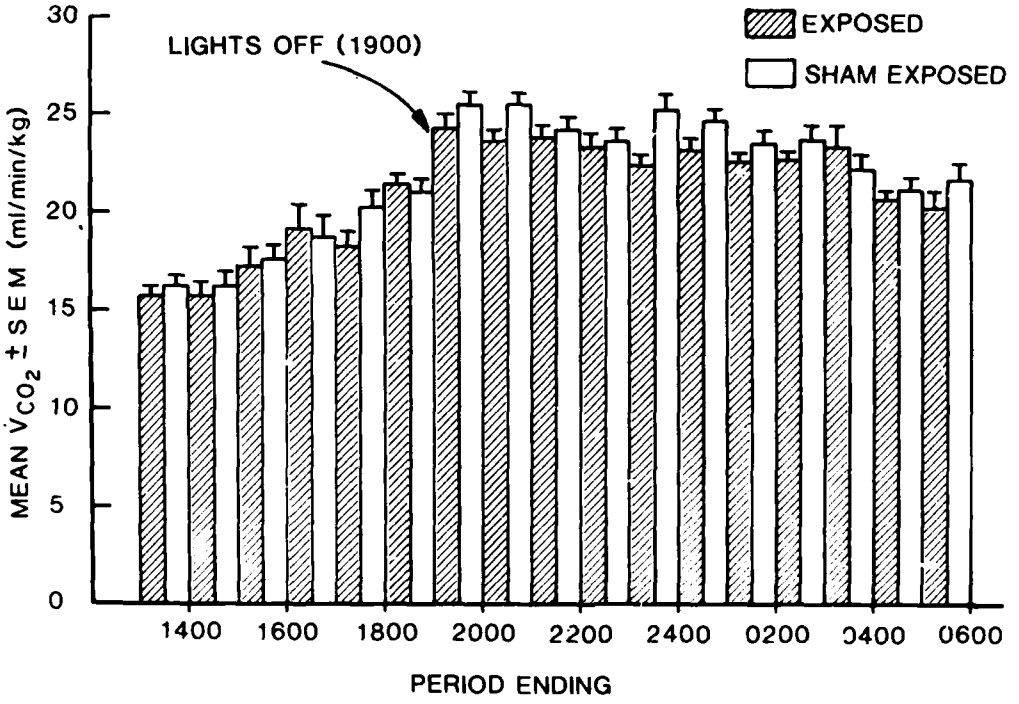

Figure 26. Average hourly respiratory quotient (RQ) for young rats (RQ = carbon dioxide production/oxygen consumption). The discontinuous scale brackets the accepted RQ range.

TABLE 17. SUMMARY OF AVERAGE HOURLY RESPIRATORY QUOTIENT FOR YOUNG RATS

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	0.78	0.02	28	0.80	0.02	28	-0.65	.51	54
2	0.75	0.02	28	0.77	0.02	28	-0.58	.56	54
3	0.76	0.02	28	0.77	0.02	28	-0.44	.65	54
4	0.77	0.03	28	0.77	0.02	28	0.01	.99	54
5	0.76	0.03	28	0.79	0.02	28	-0.83	.40	54
6	0.83	0.03	28	0.81	0.03	28	0.42	.68	54
	T^2 = 13.26			F = 2.00		p = .08		df = 6,49	
7	0.84	0.03	28	0.86	0.04	28	-0.36	.71	54
8	0.82	0.02	28	0.84	0.03	28	-0.48	.63	54
9	0.83	0.03	28	0.84	0.03	28	-0.19	.85	54
10	0.81	0.03	28	0.82	0.03	28	-0.15	.88	54
11	0.79	0.03	28	0.83	0.03	28	-0.84	.41	54
12	0.81	0.03	28	0.83	0.03	28	-0.26	.79	54
13	0.80	0.03	28	0.82	0.04	28	-0.31	.89	54
14	0.80	0.03	28	0.82	0.03	28	-0.14	.89	54
15	0.80	0.03	28	0.79	0.04	28	0.44	.66	54
16	0.75	0.04	28	0.75	0.04	28	0.06	.95	54
17	0.72	0.04	28	0.74	0.05	28	-0.17	.87	54
	T^2 = 23.8			F = 1.76		p = .09		df = 11,44	

The ratios of average hourly maximum/minimum oxygen consumption are presented in Fig. 27 and Table 18 for the young animals. The data suggest that the exposed animals tend to exhibit a lower metabolism quotient than the sham exposed. This is supported by multiple significant t-test comparisons during the diurnal period and the Hotelling T^2 statistic (F = 5.68, p = .0002, df = 6,49). The effect appears somewhat less pronounced during the nighttime hours but is still supported by multiple significant t-test comparisons and the multivariate T^2 statistic (F = 3.56, p = .001, df = 11,44).

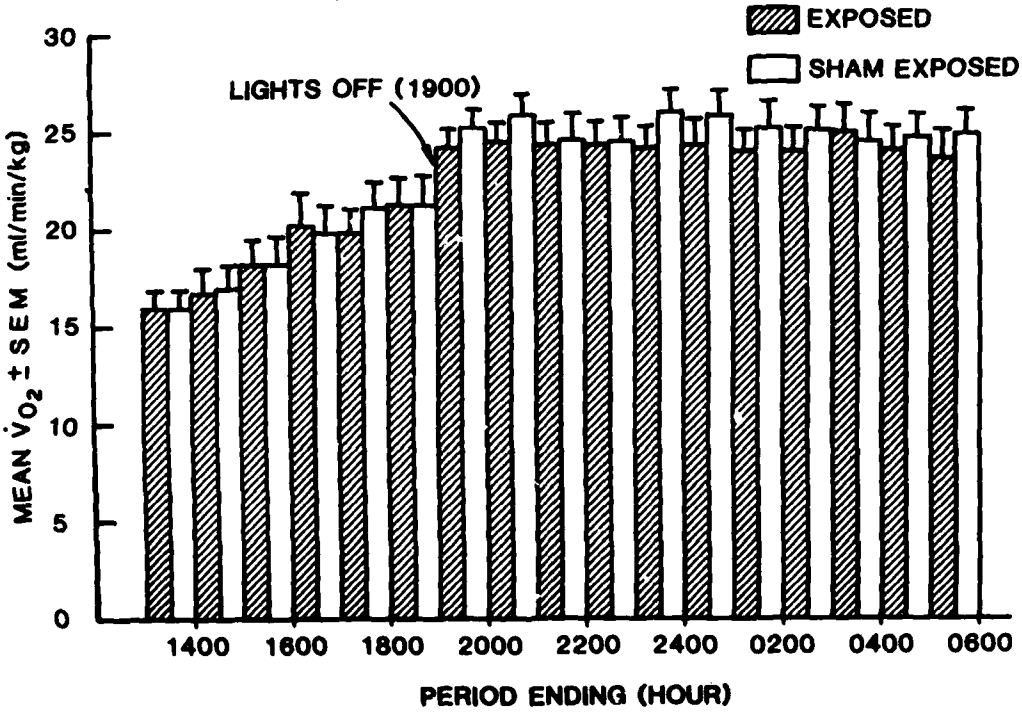

Figure 27. Average hourly metabolism quotient (MQ) for young rats (MQ = maximum/minimum oxygen consumption). The discontinuous scale emphasizes minor differences between treatment conditions.

TABLE 18. SUMMARY OF AVERAGE HOURLY METABOLISM QUOTIENT FOR YOUNG RATS

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	1.51	0.05	28	1.57	0.07	28	-0.68	.50	54
2	1.38	0.02	28	1.57	0.05	28	-3.65 *	.001	54
3	1.50	0.03	28	1.42	0.02	28	1.92	.06	54
4	1.51	0.03	28	1.65	0.08	28	-1.67	.10	54
5	1.54	0.06	28	1.76	0.07	28	-2.40 *	.02	54
6	1.73	0.06	28	1.75	0.09	28	-0.18	.86	54
	T^2 = 37.56 F = 5.68 p = .0002 df = 6,49 *								
7	1.69	0.05	28	1.83	0.06	28	-1.66	.11	54
8	1.63	0.04	28	1.78	0.06	28	-2.01 *	.05	54
9	1.63	0.04	28	1.78	0.07	28	-2.05 *	.04	54
10	1.67	0.04	28	1.76	0.07	28	-1.10	.27	54
11	1.67	0.05	28	1.75	0.06	28	-0.96	.34	54
12	1.72	0.07	28	1.80	0.08	28	-0.90	.37	54
13	1.77	0.06	28	1.72	0.06	28	0.58	.56	54
14	1.73	0.06	28	1.81	0.08	28	-0.76	.45	54
15	1.75	0.08	28	1.82	0.11	28	-0.53	.60	54
16	1.63	0.07	28	1.74	0.08	28	-1.04	.30	54
17	1.69	0.08	28	1.74	0.09	28	-0.42	.67	54
	T^2 = 47.99 F = 3.56 p = .001 df = 11,44 *								

*Statistically significant.

The average hourly rise in metabolism-cage temperature is presented for the young animals in Fig. 28 and Table 19. A perceptible elevation in cage temperature is coincident with the lights-off condition; and, in general, the exposed animals appear to have 0.1-0.2°C lower cage temperatures than the sham-exposed animals. During the daytime hours, all but one t-test comparison indicate that the hourly differences between groups are significant. However, the T^2 statistic is of only marginal significance ($F = 2.08$, $p = .07$, df = 6,49). Although only a few of the nighttime t-test comparisons indicate significant differences, the T^2 statistic indicates a highly significant overall nocturnal difference between the groups ($F = 5.59$, $p < .0001$, df = 11,44).

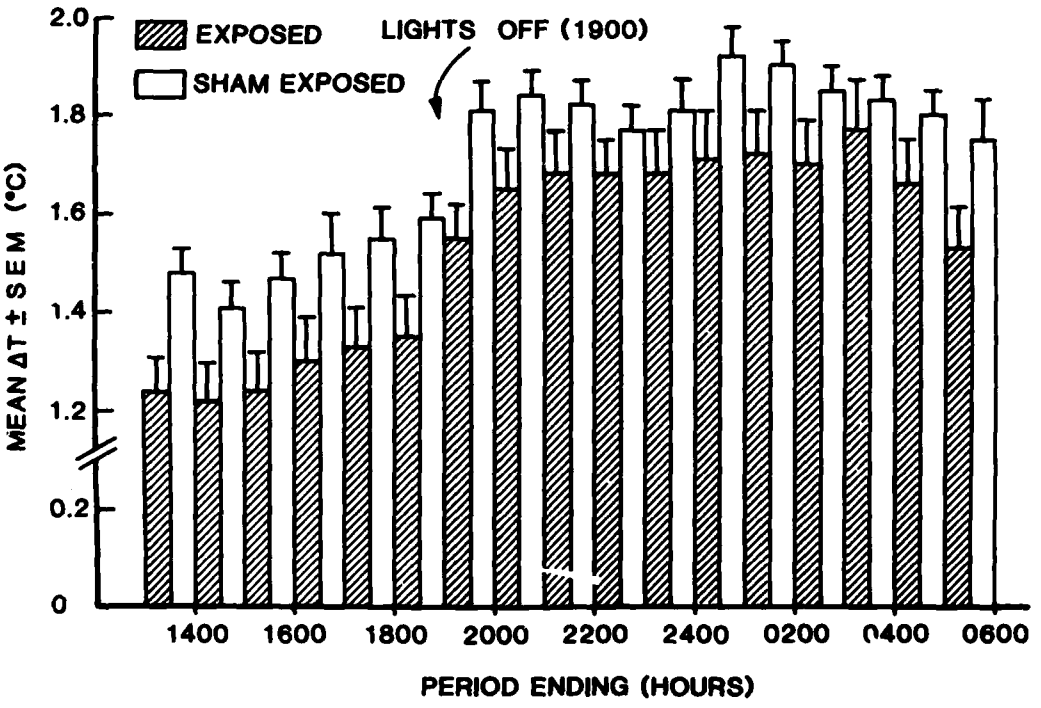

Figure 28. Average hourly cage temperature rise for young rats ($T_{delta} = T_{exhaust} - T_{intake}$). The discontinuous scale emphasizes minor differences between the treatment conditions.

TABLE 19. SUMMARY OF AVERAGE HOURLY CAGE TEMPERATURE RISE FOR YOUNG RATS (^{o}C)

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	1.24	0.08	28	1.50	0.05	28	-2.74 *	.001	54
2	1.22	0.08	28	1.41	0.05	28	-2.16 *	.04	54
3	1.24	0.08	28	1.47	0.08	28	-2.28 *	.02	54
4	1.30	0.09	28	1.52	0.07	28	-1.87	.07	54
5	1.32	0.08	28	1.54	0.07	28	-2.10 *	.04	54
6	1.35	0.08	28	1.59	0.04	28	-2.61 *	.01	54
	T^2 = 13.73			F = 2.08			p = .07	df = 6,49	
7	1.55	0.07	28	1.81	0.06	28	-2.88 *	.01	54
8	1.65	0.08	28	1.84	0.04	28	-2.05 *	.04	54
9	1.68	0.09	28	1.82	0.05	28	-1.39	.16	54
10	1.68	0.07	28	1.77	0.04	28	-1.11	.27	54
11	1.69	0.09	28	1.80	0.06	28	-1.14	.26	54
12	1.71	0.10	28	1.92	0.05	28	-1.77	.08	54
13	1.72	0.09	28	1.90	0.04	28	-1.85	.07	54
14	1.70	0.08	28	1.85	0.04	28	-1.51	.14	54
15	1.77	0.10	28	1.82	0.06	28	-0.48	.63	54
16	1.66	0.08	28	1.80	0.05	28	-1.34	.18	54
17	1.52	0.08	28	1.75	0.08	28	-2.00 *	.05	54
	T^2 = 75.43			F = 5.59			p < .0001	df = 11,44 *	

*Statistically significant.

The second analysis of the data was completed using only one 17-h sample from each surviving animal during a single round of measurements. Analyses were completed for four rounds of measurement for the mature animals, but summaries of only the first two are presented in Tables 20-29. Similar results were obtained from analysis of the other rounds.

None of the t-test comparisons during the first or second round of measurements for the mature animals indicates significant differences between treatment conditions on the measures of oxygen consumption (Tables 20 and 21), carbon dioxide production (Tables 22 and 23), respiratory quotient (Tables 24 and 25), or metabolism quotient (Tables 26 and 27). The multivariate analyses of the diurnal and nocturnal periods also indicate no differences. Multiple significant t-tests indicate an elevation in the exposed-animal cage temperature rise during the first round (Table 28), but this is not supported by the multivariate analysis of either the diurnal or nocturnal periods; no significant differences are indicated in the second round (Table 29).

Although only a few t-test comparisons indicate that the young exposed animals had significantly lower levels of oxygen consumption (Tables 30 and 31) and carbon dioxide production (Tables 32 and 33) and lower RQ values (Tables 34 and 35), the multivariate analyses indicate these differences to be significant during both diurnal and nocturnal periods of the first round. Multiple univariate t-tests and the multivariate analyses indicate lower metabolism quotients for the exposed animals during diurnal and nocturnal periods of both the first and second round of measurement (Tables 36 and 37). The multivariate analyses indicate a lower cage-temperature rise during the nocturnal periods of both the first and second round of measurements (Tables 38 and 39).

TABLE 20. SUMMARY OF ROUND-ONE AVERAGE HOURLY OXYGEN CONSUMPTION FOR MATURE RATS (ml/min per kg)

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	16.21	0.96	12	16.81	0.77	13	-0.49	.63	23
2	16.70	1.13	12	16.12	0.80	13	0.43	.67	23
3	16.41	1.32	12	16.46	0.74	13	-0.03	.97	23
4	17.10	1.33	12	17.04	0.55	13	0.04	.97	23
5	16.71	1.04	12	16.86	0.69	13	-0.12	.91	23
6	18.46	1.23	12	17.32	0.85	13	0.78	.45	23
	$T^2 = 4.79$ $F = 0.62$ $p = .71$ $df = 6,18$								
7	18.59	0.92	12	18.09	0.66	13	0.45	.66	23
8	19.58	0.94	12	18.32	0.65	13	1.12	.28	23
9	20.08	1.14	12	19.40	0.92	13	0.46	.65	23
10	19.56	1.15	12	19.45	1.04	13	0.07	.95	23
11	19.38	0.91	12	19.58	1.20	13	-0.13	.89	23
12	18.58	1.10	12	18.52	0.85	13	0.04	.97	23
13	19.04	1.32	12	18.85	1.24	13	0.11	.92	23
14	20.34	1.47	12	20.08	1.22	13	0.14	.89	23
15	18.76	1.01	12	18.69	1.08	13	0.05	.96	23
16	20.80	1.18	12	20.05	0.97	13	0.50	.62	23
17	20.48	1.44	12	19.69	0.97	13	0.46	.65	23
	$T^2 = 6.77$ $F = 0.35$ $p = .96$ $df = 11,13$								

TABLE 21. SUMMARY OF ROUND-TWO AVERAGE HOURLY OXYGEN CONSUMPTION FOR MATURE RATS (ml/min per kg)

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	21.79	5.45	11	15.61	0.67	12	1.18	.25	21
2	23.27	5.47	11	16.31	0.65	12	1.32	.20	21
3	23.20	6.03	11	16.25	0.70	12	1.20	.24	21
4	23.83	5.66	11	15.69	0.68	12	1.49	.15	21
5	23.56	5.49	11	16.28	0.57	12	1.38	.18	21
6	25.92	6.41	11	17.63	0.97	12	1.33	.20	21
	T^2 = 12.86 F = 1.63 p = .20 df = 6,16								
7	25.90	4.88	11	17.40	0.83	12	1.79	.09	21
8	26.03	5.94	11	17.67	0.83	12	1.46	.16	21
9	28.65	7.08	11	19.74	0.98	12	1.30	.21	21
10	28.20	6.93	11	18.02	1.03	12	1.52	.14	21
11	30.54	8.07	11	18.27	1.26	12	1.57	.13	21
12	29.70	7.47	11	18.89	1.12	12	1.49	.15	21
13	31.32	9.95	11	19.02	1.26	12	1.28	.21	21
14	33.43	10.86	11	19.98	1.28	12	1.29	.21	21
15	32.96	9.02	11	20.85	1.90	12	1.37	.18	21
16	35.38	10.79	11	23.16	1.88	12	1.17	.26	21
17	35.23	10.91	11	22.86	2.00	12	1.16	.26	21
	T^2 = 45.02 F = 2.14 p = .11 df = 11,11								

TABLE 22. SUMMARY OF ROUND-ONE AVERAGE HOURLY CARBON DIOXIDE PRODUCTION FOR MATURE RATS (ml/min per kg)

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	13.78	0.66	12	13.96	0.59	13	-0.20	.84	23
2	14.32	0.77	12	13.47	0.61	13	0.88	.39	23
3	14.03	0.85	12	13.65	0.44	13	0.41	.68	23
4	14.76	1.03	12	14.34	0.44	13	0.39	.70	23
5	14.19	0.63	12	14.07	0.47	13	0.16	.88	23
6	15.42	0.82	12	14.26	0.65	13	1.12	.27	23
	$T^2 = 2.33$ $F = 0.30$ $p = .93$ df = 6,18								
7	15.52	0.67	12	14.87	0.70	13	0.67	.51	23
8	16.34	0.80	12	14.76	0.42	13	1.78	.09	23
9	16.80	0.43	12	15.64	0.59	13	1.58	.13	23
10	16.22	0.53	12	15.45	0.77	13	0.81	.42	23
11	16.40	0.34	12	15.79	0.90	13	0.62	.54	23
12	16.00	0.71	12	14.97	0.76	13	0.99	.33	23
13	16.50	0.82	12	15.08	0.94	13	1.13	.27	23
14	17.19	0.82	12	15.88	0.94	13	1.04	.31	23
15	15.49	0.58	12	14.60	0.49	13	1.18	.25	23
16	16.78	0.60	12	15.71	0.87	13	1.00	.33	23
17	16.31	0.80	12	15.27	1.02	13	0.79	.44	23
	$T^2 = 10.98$ $F = 0.56$ $p = .82$ df = 11,13								

TABLE 23. SUMMARY OF ROUND-TWO AVERAGE HOURLY CARBON DIOXIDE PRODUCTION FOR MATURE RATS (ml/min per kg)

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	17.86	5.02	11	13.42	0.56	12	0.92	.37	21
2	18.55	4.71	11	14.11	0.55	12	0.98	.34	21
3	18.04	4.77	11	14.34	0.61	12	0.80	.43	21
4	18.40	4.40	11	13.84	0.73	12	1.07	.30	21
5	18.29	4.34	11	14.29	0.41	12	0.96	.35	21
6	20.65	5.54	11	15.43	0.73	12	0.98	.34	21
	$T^2 = 5.34$ $F = 0.68$ $p = .67$ $df = 6,16$								
7	20.45	4.55	11	15.09	0.68	12	1.22	.24	21
8	20.10	5.00	11	15.17	0.56	12	1.03	.32	21
9	22.35	5.96	11	17.08	0.90	12	0.91	.37	21
10	21.74	5.81	11	15.44	0.92	12	1.12	.28	21
11	23.08	6.07	11	15.71	0.87	12	1.25	.22	21
12	21.83	5.05	11	16.32	0.85	12	1.12	.27	21
13	22.21	6.74	11	16.16	0.78	12	0.93	.36	21
14	22.95	7.18	11	16.08	0.68	12	1.00	.33	21
15	21.81	5.37	11	16.04	0.87	12	1.11	.28	21
16	22.83	6.63	11	17.44	0.85	12	0.84	.41	21
	$T^2 = 21.57$ $F = 1.03$ $p = .48$ $df = 11,11$								

TABLE 24. SUMMARY OF ROUND-ONE AVERAGE HOURLY RESPIRATORY QUOTIENT FOR MATURE RATS

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	0.86	0.03	12	0.84	0.03	13	0.54	.60	23
2	0.87	0.03	12	0.84	0.03	13	0.67	.51	23
3	0.87	0.03	12	0.84	0.03	13	0.82	.42	23
4	0.87	0.03	12	0.85	0.03	13	0.67	.51	23
5	0.86	0.03	12	0.84	0.03	13	0.65	.53	23
6	0.85	0.03	12	0.83	0.03	13	0.40	.69	23
	T^2 = 1.08 F = 0.14 p = .99 df = 6,18								
7	0.84	0.03	12	0.82	0.04	13	0.33	.74	23
8	0.84	0.04	12	0.81	0.04	13	0.52	.61	23
9	0.85	0.04	12	0.82	0.04	13	0.71	.48	23
10	0.85	0.04	12	0.81	0.04	13	0.73	.47	23
11	0.86	0.04	12	0.82	0.04	13	0.72	.48	23
12	0.88	0.05	12	0.82	0.05	13	0.87	.39	23
13	0.88	0.04	12	0.82	0.04	13	1.06	.30	23
14	0.87	0.04	12	0.81	0.04	13	0.98	.34	23
15	0.84	0.04	12	0.80	0.04	13	0.64	.53	23
16	0.82	0.03	12	0.80	0.04	13	0.35	.73	23
17	0.81	0.03	12	0.78	0.04	13	0.49	.63	23
	T^2 = 13.18 F = 0.68 p = .74 df = 11,13								

TABLE 25. SUMMARY OF ROUND-TWO AVERAGE HOURLY RESPIRATORY QUOTIENT FOR MATURE RATS

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	0.82	0.05	11	0.87	0.04	12	-0.71	.49	21
2	0.80	0.05	11	0.87	0.04	12	-1.20	.24	21
3	0.79	0.04	11	0.89	0.04	12	-1.73	.10	21
4	0.79	0.04	11	0.89	0.04	12	-1.68	.11	21
5	0.79	0.05	11	0.89	0.04	12	-1.52	.14	21
6	0.80	0.05	11	0.89	0.05	12	-1.30	.21	21
	T^2 = 10.80 F = 1.37 p = .28 df = 6,16								
7	0.78	0.05	11	0.88	0.05	12	-1.45	.16	21
8	0.77	0.06	11	0.88	0.06	12	-1.32	.20	21
9	0.78	0.05	11	0.88	0.05	12	-1.35	.19	21
10	0.76	0.05	11	0.87	0.05	12	-1.51	.14	21
11	0.76	0.04	11	0.88	0.04	12	-1.89	.07	21
12	0.76	0.04	11	0.88	0.06	12	-1.82	.08	21
13	0.73	0.03	11	0.88	0.06	12	-2.21	.04	21
14	0.70	0.04	11	0.83	0.06	12	-1.94	.07	21
15	0.69	0.04	11	0.82	0.06	12	-1.85	.08	21
16	0.67	0.03	11	0.80	0.06	12	-1.90	.07	21
17	0.66	0.04	11	0.78	0.05	12	-1.71	.10	21
	T^2 = 24.19 F = 1.15 p = .41 df = 11,11								

TABLE 26. SUMMARY OF ROUND-ONE AVERAGE HOURLY METABOLISM QUOTIENT FOR MATURE RATS

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	1.75	0.08	12	1.79	0.08	13	-0.29	.77	23
2	1.77	0.08	12	1.71	0.07	13	0.54	.60	23
3	1.61	0.09	12	1.74	0.10	13	-0.92	.37	23
4	1.87	0.08	12	1.77	0.08	13	0.89	.38	23
5	1.82	0.07	12	1.68	0.06	13	1.53	.14	23
6	1.88	0.08	12	1.72	0.07	13	1.44	.16	23
	T^2 = 9.78 F = 1.28 p = .32 df = 6,18								
7	1.84	0.10	12	1.76	0.10	13	0.61	.54	23
8	1.91	0.08	12	1.87	0.09	13	0.34	.74	23
9	1.89	0.10	12	1.92	0.10	13	-0.21	.83	23
10	1.89	0.09	12	1.82	0.14	13	0.42	.68	23
11	1.99	0.10	12	1.80	0.10	13	1.45	.16	23
12	1.84	0.10	12	1.79	0.12	13	0.34	.74	23
13	1.85	0.08	12	1.74	0.11	13	0.85	.40	23
14	1.89	0.08	12	1.86	0.12	13	0.18	.86	23
15	1.83	0.13	12	1.73	0.08	13	0.68	.50	23
16	1.82	0.10	12	1.79	0.12	13	0.22	.83	23
17	1.73	0.08	12	1.69	0.09	13	0.32	.75	23
	T^2 = 5.88 F = 0.30 p = .97 df = 11,13								

TABLE 27. SUMMARY OF ROUND-TWO AVERAGE HOURLY METABOLISM QUOTIENT FOR MATURE RATS

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	1.70	0.17	11	1.66	0.09	12	0.19	.85	21
2	1.68	0.08	11	1.97	0.15	12	-1.66	.11	21
3	1.56	0.06	11	1.86	0.09	12	-2.58	.02	21
4	1.67	0.05	11	1.72	0.09	12	-0.45	.66	21
5	1.53	0.08	11	1.74	0.10	12	-1.62	.12	21
6	1.71	0.07	11	1.91	0.10	12	-1.58	.13	21
	T^2 = 12.46 F = 1.58 p = .22 df = 6,16								
7	1.94	0.13	11	1.98	0.06	12	-0.29	.77	21
8	1.78	0.08	11	2.17	0.16	12	-2.16 *	.04	21
9	1.84	0.05	11	1.97	0.15	12	-0.78	.44	21
10	1.99	0.12	11	1.79	0.09	12	1.36	.19	21
11	1.86	0.07	11	2.04	0.18	12	-1.14	.27	21
12	1.82	0.05	11	1.99	0.07	12	-1.78	.09	21
13	1.66	0.07	11	1.95	0.09	12	-2.45 *	.02	21
14	1.64	0.08	11	1.98	0.15	12	-1.92	.07	21
15	1.73	0.08	11	1.76	0.08	12	-0.32	.75	21
16	1.68	0.05	11	1.84	0.08	12	-1.58	.13	21
17	1.66	0.06	11	1.80	0.08	12	-1.37	.18	21
	T^2 = 66.21 F = 3.15 p = .04 df = 11,11 *								

*statistically significant.

TABLE 28. SUMMARY OF ROUND-ONE AVERAGE HOURLY CAGE TEMPERATURE RISE FOR MATURE RATS

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	1.62	0.06	12	1.48	0.09	13	1.26	.22	23
2	1.68	0.05	12	1.46	0.08	13	2.29 *	.03	23
3	1.67	0.07	12	1.41	0.08	13	2.40 *	.02	23
4	1.67	0.08	12	1.45	0.06	13	2.36 *	.03	23
5	1.64	0.07	12	1.44	0.05	13	2.40 *	.02	23
6	1.66	0.07	12	1.42	0.06	13	2.60 *	.02	23
	T^2 = 9.79 F = 1.28 p = .32 df = 6,18								
7	1.68	0.05	12	1.45	0.06	13	3.02 *	.01	23
8	1.70	0.06	12	1.51	0.06	13	2.20 *	.04	23
9	1.74	0.08	12	1.54	0.05	13	2.16 *	.04	23
10	1.76	0.05	12	1.63	0.07	13	1.55	.13	23
11	1.76	0.07	12	1.65	0.09	13	0.89	.38	23
12	1.69	0.07	12	1.61	0.10	13	0.64	.53	23
13	1.70	0.06	12	1.60	0.07	13	0.91	.37	23
14	1.77	0.07	12	1.60	0.08	13	1.51	.14	23
15	1.76	0.08	12	1.53	0.06	13	2.32 *	.03	23
16	1.75	0.09	12	1.54	0.06	13	2.00	.06	23
17	1.76	0.07	12	1.58	0.06	13	1.99	.06	23
	T^2 = 17.63 F = 0.91 p = .56 df = 11,13								

*Statistically significant.

TABLE 29. SUMMARY OF ROUND-TWO AVERAGE HOURLY CAGE TEMPERATURE RISE FOR MATURE RATS

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	1.57	0.09	11	1.60	0.08	12	-0.24	.81	21
2	1.63	0.05	11	1.70	0.10	12	-0.64	.53	21
3	1.67	0.07	11	1.78	0.09	12	-1.01	.32	21
4	1.63	0.08	11	1.70	0.10	12	-0.57	.58	21
5	1.62	0.07	11	1.70	0.10	12	-0.66	.52	21
6	1.69	0.08	11	1.73	0.09	12	-0.31	.76	21
	$T^2 = 2.87$ $F = 0.36$ $p = .89$ $df = 6,16$								
7	1.78	0.05	11	1.81	0.09	12	-0.29	.77	21
8	1.84	0.06	11	1.81	0.09	12	0.28	.79	21
9	1.76	0.04	11	1.84	0.10	12	-0.72	.48	21
10	1.76	0.04	11	1.84	0.10	12	-0.33	.74	21
11	1.81	0.06	11	1.84	0.09	12	-0.24	.81	21
12	1.89	0.08	11	1.86	0.11	12	0.20	.84	21
13	1.76	0.08	11	1.94	0.10	12	-1.40	.18	21
14	1.79	0.09	11	1.82	0.11	12	-0.23	.82	21
15	1.84	0.09	11	1.89	0.12	12	-0.32	.76	21
16	1.84	0.09	11	1.95	0.14	12	-0.66	.51	21
17	1.83	0.08	11	1.89	0.10	12	-0.44	.66	21
	$T^2 = 33.27$ $F = 1.58$ $p = .23$ $df = 11,11$								

TABLE 30. SUMMARY OF ROUND-ONE AVERAGE HOURLY OXYGEN CONSUMPTION FOR YOUNG RATS (ml/min per kg)

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	23.37	1.22	17	24.14	0.91	15	-0.49	.63	30
2	25.07	1.79	17	25.29	1.54	15	-0.09	.93	30
3	26.35	1.93	17	26.43	1.86	15	-0.03	.97	30
4	28.27	2.15	17	27.14	2.20	15	0.37	.72	30
5	26.23	1.72	17	27.56	1.61	15	-0.56	.58	30
6	27.41	1.57	17	27.72	1.62	15	-0.16	.87	30
	T^2 = 26.79			F = 3.72			p = .009	df = 6,25 *	
7	30.44	1.11	17	30.79	1.23	15	-0.21	.83	30
8	29.30	1.43	17	30.31	0.93	15	-0.58	.57	30
9	29.21	1.39	17	29.30	1.06	15	-0.05	.96	30
10	29.85	1.39	17	29.46	1.36	15	0.20	.84	30
11	29.12	1.38	17	30.92	1.25	15	-0.96	.35	30
12	29.13	1.65	17	29.96	1.54	15	-0.37	.72	30
13	28.77	1.47	17	29.47	1.70	15	-0.31	.76	30
14	29.24	1.77	17	29.44	1.84	15	-0.08	.94	30
15	30.93	1.93	17	28.61	1.54	15	0.92	.36	30
16	29.46	1.47	17	29.11	1.41	15	0.17	.87	30
17	28.79	1.89	17	29.08	1.80	15	-0.11	.91	30
	T^2 = 50.43			F = 3.06			p = .015	df = 11,20	

*Statistically significant.

TABLE 31. SUMMARY OF ROUND-TWO AVERAGE HOURLY OXYGEN CONSUMPTION FOR YOUNG RATS (ml/min per kg)

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	17.18	0.63	10	16.59	0.66	10	0.65	.52	18
2	16.51	0.69	10	16.66	0.91	10	-0.13	.90	18
3	18.06	0.98	10	17.67	1.26	10	0.24	.81	18
4	19.91	1.61	10	19.50	1.27	10	0.20	.85	18
5	22.20	1.98	10	22.51	2.38	10	-0.10	.92	18
6	24.84	2.32	10	23.61	2.24	10	0.38	.71	18
	T^2 = 12.06 F = 1.45 p = .27 df = 6,13								
7	27.04	2.03	10	28.45	2.33	10	-0.46	.65	18
8	29.03	2.32	10	30.10	2.31	10	-0.33	.75	18
9	29.09	2.19	10	28.36	3.28	10	0.18	.86	18
10	28.10	2.34	10	28.19	2.57	10	-0.02	.98	18
11	28.80	2.57	10	30.17	2.70	10	-0.37	.72	18
12	29.15	2.72	10	30.61	2.90	10	-0.37	.72	18
13	29.19	2.20	10	29.43	3.28	10	-0.06	.95	18
14	28.01	2.67	10	29.49	1.92	10	-0.45	.66	18
15	27.59	2.76	10	28.86	2.90	10	-0.32	.75	18
16	27.27	2.07	10	28.35	2.94	10	-0.26	.80	18
17	27.45	2.92	10	29.63	2.66	10	-0.55	.59	18
	T^2 = 119.49 F = 4.83 p = .02 df = 11,8 *								

*Statistically significant.

TABLE 32. SUMMARY OF ROUND-ONE AVERAGE HOURLY CARBON DIOXIDE PRODUCTION FOR YOUNG RATS (ml/min per kg)

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	16.55	0.75	17	17.39	0.67	15	-0.83	.41	30
2	17.15	1.03	17	17.81	0.82	15	-0.49	.62	30
3	19.55	1.28	17	19.64	1.12	15	-0.05	.96	30
4	22.36	1.44	17	20.90	1.47	15	0.71	.49	30
5	20.14	0.89	17	22.70	0.99	15	-1.93	.06	30
6	22.80	0.92	17	23.52	0.83	15	-0.58	.57	30
	T^2 = 35.41 F = 4.92 p = .002 df = 6,25 *								
7	26.41	0.83	17	27.11	0.76	15	-0.62	.54	30
8	24.03	0.86	17	26.03	0.82	15	-1.68	.10	30
9	24.76	0.83	17	25.36	0.66	15	-0.56	.58	30
10	25.20	0.74	17	25.20	0.91	15	0.00	1.00	30
11	23.53	0.74	17	27.11	0.72	15	-3.44 *	.00	30
12	23.93	1.12	17	25.32	0.96	15	-0.93	.36	30
13	23.02	0.69	17	24.34	1.21	15	-0.98	.33	30
14	23.95	0.84	17	24.85	1.10	15	-0.66	.52	30
15	25.12	1.43	17	22.02	0.41	15	1.97	.06	30
16	20.95	0.58	17	21.19	0.48	15	-0.32	.75	30
17	20.80	1.54	17	22.16	1.80	15	-0.57	.57	30
	T^2 = 78.51 F = 4.76 p = .001 df = 11,20 *								

*Statistically significant.

TABLE 33. SUMMARY OF ROUND-TWO AVERAGE HOURLY CARBON DIOXIDE PRODUCTION FOR YOUNG RATS (ml/min per kg)

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	14.85	0.59	10	14.49	0.52	10	0.46	.65	18
2	13.56	0.33	10	13.90	0.46	10	-0.60	.55	18
3	14.00	0.42	10	14.31	0.60	10	-0.43	.67	18
4	14.18	0.58	10	15.17	0.43	10	-1.38	.18	18
5	15.56	0.54	10	17.02	1.19	10	-1.12	.28	18
6	19.56	0.39	10	17.76	0.40	10	3.23 *	.00	18
	T^2 = 40.34			F = 4.86			p = .008	df = 6,13 *	
7	21.06	0.85	10	23.79	0.53	10	-2.72 *	.01	18
8	22.96	0.72	10	24.45	0.98	10	-1.29	.21	18
9	22.51	0.63	10	22.47	1.23	10	0.03	.98	18
10	20.46	0.70	10	21.61	0.68	10	-1.18	.26	18
11	20.93	0.54	10	23.36	1.00	10	-2.13 *	.05	18
12	22.00	0.62	10	23.98	0.59	10	-2.32 *	.03	18
13	21.94	0.94	10	22.36	1.28	10	-0.26	.80	18
14	20.64	0.50	10	22.38	1.23	10	-1.31	.21	18
15	20.64	0.45	10	21.84	1.90	10	-0.61	.55	18
16	19.87	0.98	10	20.89	1.23	10	-0.65	.52	18
17	19.27	0.37	10	21.72	0.40	10	-4.49 *	.00	18
	T^2 = 52.27			F = 2.11			p = .15	df = 11,8 *	

*Statistically significant.

TABLE 34. SUMMARY OF ROUND-ONE AVERAGE HOURLY RESPIRATORY QUOTIENT FOR YOUNG RATS

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	0.72	0.03	17	0.72	0.03	15	-0.14	.89	30
2	0.70	0.02	17	0.71	0.03	15	-0.47	.64	30
3	0.75	0.02	17	0.75	0.03	15	-0.11	.91	30
4	0.80	0.02	17	0.78	0.02	15	0.73	.47	30
5	0.78	0.02	17	0.83	0.03	15	-1.21	.24	30
6	0.84	0.03	17	0.86	0.03	15	-0.32	.75	30
	T^2 = 25.61 F = 3.56 p = .011 df = 6,25 *								
7	0.88	0.03	17	0.89	0.03	15	-0.22	.82	30
8	0.82	0.02	17	0.86	0.03	15	-0.96	.35	30
9	0.86	0.03	17	0.87	0.03	15	-0.33	.74	30
10	0.85	0.03	17	0.86	0.02	15	-0.16	.88	30
11	0.82	0.03	17	0.88	0.03	15	-1.49	.15	30
12	0.83	0.03	17	0.86	0.04	15	-0.53	.60	30
13	0.81	0.03	17	0.84	0.04	15	-0.54	.60	30
14	0.83	0.03	17	0.86	0.04	15	-0.52	.61	30
15	0.82	0.03	17	0.80	0.05	15	0.42	.68	30
16	0.73	0.04	17	0.74	0.05	15	-0.27	.79	30
17	0.71	0.05	17	0.75	0.06	15	-0.43	.67	30
	T^2 = 65.55 F = 3.97 p = .004 df = 11,20 *								

*Statistically significant.

TABLE 35. SUMMARY OF ROUND-TWO AVERAGE HOURLY RESPIRATORY QUOTIENT FOR YOUNG RATS

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	0.87	0.03	10	0.88	0.04	10	-0.26	.80	18
2	0.84	0.04	10	0.85	0.04	10	-0.26	.80	18
3	0.79	0.04	10	0.83	0.04	10	-0.65	.52	18
4	0.74	0.04	10	0.80	0.04	10	-0.86	.40	18
5	0.74	0.06	10	0.78	0.04	10	-0.58	.57	18
6	0.84	0.07	10	0.80	0.07	10	0.33	.75	18
	T^2 = 14.98			F = 1.80			p = .18	df = 6,13	
7	0.82	0.07	10	0.87	0.08	10	-0.63	.53	18
8	0.83	0.07	10	0.85	0.08	10	-0.22	.83	18
9	0.82	0.08	10	0.85	0.06	10	-0.29	.77	18
10	0.78	0.08	10	0.82	0.08	10	-0.38	.71	18
11	0.78	0.07	10	0.82	0.08	10	-0.43	.67	18
12	0.82	0.08	10	0.84	0.08	10	-0.20	.84	18
13	0.80	0.08	10	0.82	0.07	10	-0.23	.82	18
14	0.79	0.07	10	0.80	0.09	10	-0.15	.88	18
15	0.80	0.08	10	0.81	0.08	10	-0.02	.99	18
16	0.80	0.08	10	0.79	0.08	10	0.04	.96	18
17	0.78	0.08	10	0.80	0.09	10	-0.17	.87	18
	T^2 = 53.32			F = 2.67			p = .078	df = 10,9	

TABLE 36. SUMMARY OF ROUND-ONE AVERAGE HOURLY METABOLISM QUOTIENT FOR YOUNG RATS

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	1.38	0.04	17	1.43	0.07	15	-0.74	.46	30
2	1.40	0.03	17	1.55	0.07	15	-2.17 *	.04	30
3	1.48	0.04	17	1.43	0.03	15	1.05	.30	30
4	1.55	0.03	17	1.47	0.03	15	1.56	.13	30
5	1.49	0.04	17	1.77	0.11	15	-2.49 *	.02	30
6	1.66	0.06	17	1.68	0.07	15	-0.20	.84	30
	T^2 = 20.94			F = 2.91			p = .027	df = 6,25 *	
7	1.62	0.06	17	1.84	0.08	15	-2.11 *	.04	30
8	1.57	0.02	17	1.73	0.06	15	-2.47 *	.02	30
9	1.58	0.03	17	1.84	0.10	15	-2.70 *	.01	30
10	1.62	0.05	17	1.79	0.08	15	-1.80	.08	30
11	1.63	0.06	17	1.87	0.07	15	-2.69 *	.01	30
12	1.63	0.06	17	1.77	0.08	15	-1.40	.17	30
13	1.75	0.07	17	1.70	0.09	15	0.53	.60	30
14	1.64	0.05	17	1.89	0.10	15	-2.30 *	.03	30
15	1.66	0.07	17	1.74	0.10	15	-0.76	.45	30
16	1.63	0.11	17	1.77	0.13	15	-0.81	.42	30
17	1.69	0.12	17	1.74	0.13	15	-0.24	.81	30
	T^2 = 137.80			F = 8.35			p = .0000	df = 11,20 *	

*Statistically significant.

TABLE 37. SUMMARY OF ROUND-TWO AVERAGE HOURLY METABOLISM QUOTIENT FOR YOUNG RATS

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	1.72	0.10	10	1.78	0.12	10	-0.42	.68	18
2	1.34	0.03	10	1.60	0.08	10	-3.27 *	.00	18
3	1.53	0.04	10	1.46	0.04	10	1.22	.24	18
4	1.43	0.05	10	1.94	0.19	10	-2.61 *	.02	18
5	1.65	0.14	10	1.82	0.08	10	-1.01	.32	18
6	1.89	0.12	10	1.94	0.22	10	-0.18	.86	18
	T^2 = 76.37 F = 9.19 p = .0000 df = 6,13 *								
7	1.80	0.11	10	1.92	0.12	10	-0.71	.49	18
8	1.73	0.10	10	1.91	0.12	10	-1.13	.27	18
9	1.70	0.10	10	1.78	0.11	10	-0.60	.56	18
10	1.76	0.07	10	1.80	0.13	10	-0.28	.79	18
11	1.78	0.11	10	1.72	0.09	10	0.43	.67	18
12	1.86	0.15	10	1.94	0.16	10	-0.40	.74	18
13	1.82	0.13	10	1.80	0.11	10	0.09	.93	18
14	1.91	0.15	10	1.80	0.16	10	0.51	.62	18
15	1.92	0.18	10	2.05	0.25	10	-0.43	.67	18
16	1.64	0.06	10	1.88	0.14	10	-0.96	.35	18
17	1.71	0.12	10	1.86	0.15	10	-0.96	.35	18
	T^2 = 398.35 F = 19.92 p = .0000 df = 10,9 *								

*Statistically significant.

TABLE 38. SUMMARY OF ROUND-ONE AVERAGE HOURLY CAGE TEMPERATURE RISE FOR YOUNG RATS

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	1.25	0.11	17	1.41	0.06	15	-1.24	.22	30
2	1.27	0.12	17	1.39	0.07	15	-0.84	.41	30
3	1.30	0.13	17	1.43	0.09	15	-0.80	.43	30
4	1.42	0.14	17	1.57	0.13	15	-0.82	.42	30
5	1.44	0.12	17	1.60	0.10	15	-1.03	.31	30
6	1.42	0.12	17	1.63	0.08	15	-1.47	.15	30
	$T^2 = 12.17$ $F = 1.69$ $p = .16$ df = 6,25								
7	1.64	0.11	17	1.89	0.09	15	-1.75	.09	30
8	1.74	0.12	17	1.85	0.06	15	-0.74	.47	30
9	1.73	0.14	17	1.83	0.05	15	-0.63	.53	30
10	1.73	0.11	17	1.76	0.05	15	-0.27	.79	30
11	1.76	0.14	17	1.80	0.07	15	-0.24	.81	30
12	1.77	0.16	17	1.85	0.06	15	-0.43	.67	30
13	1.73	0.14	17	1.86	0.06	15	-0.85	.40	30
14	1.68	0.13	17	1.85	0.05	15	-1.17	.25	30
15	1.77	0.16	17	1.75	0.06	15	0.13	.90	30
16	1.66	0.12	17	1.66	0.05	15	-0.01	.99	30
17	1.44	0.11	17	1.62	0.12	15	-1.03	.31	30
	$T^2 = 87.44$ $F = 5.30$ $p = .0006$ df = 11,20 *								

*Statistically significant.

TABLE 39. SUMMARY OF ROUND-TWO AVERAGE HOURLY CAGE TEMPERATURE RISE FOR YOUNG RATS

	Exposed			Sham					
H	M	SEM	N	M	SEM	N	t	p	df
1	1.20	0.09	10	1.57	0.09	10	-2.73 *	.01	18
2	1.11	0.06	10	1.40	0.06	10	-3.02 *	.01	18
3	1.13	0.08	10	1.50	0.08	10	-3.30 *	.00	18
4	1.10	0.06	10	1.41	0.08	10	-3.21 *	.00	18
5	1.14	0.07	10	1.44	0.07	10	-3.11 *	.01	18
6	1.25	0.07	10	1.52	0.05	10	-3.08 *	.01	18
	T^2 = 18.28 F = 2.20 p = .11 df = 6,13								
7	1.41	0.06	10	1.70	0.05	10	-3.90 *	.00	18
8	1.50	0.06	10	1.79	0.76	10	-3.00 *	.01	18
9	1.55	0.09	10	1.76	0.76	10	-1.83	.08	18
10	1.54	0.07	10	1.77	0.07	10	-2.43 *	.03	18
11	1.52	0.08	10	1.80	0.09	10	-2.33 *	.03	18
12	1.56	0.09	10	2.00	0.08	10	-3.55 *	.00	18
13	1.65	0.09	10	1.94	0.05	10	-2.72 *	.01	18
14	1.70	0.08	10	1.85	0.08	10	-1.24	.23	18
15	1.72	0.10	10	1.90	0.10	10	-1.21	.24	18
16	1.61	0.10	10	1.94	0.07	10	-2.72 *	.01	18
17	1.61	0.09	10	1.97	0.08	10	-3.00 *	.01	18
	T^2 = 896.79 F = 36.23 p = .0000 df = 11,8 *								

*Statistically significant.

Discussion

In general, the levels of oxygen consumption and carbon dicxide production reported here are in agreement with values reported in the literature for the rat (Kleiber, 1947; Moses, 1947; Krantzing and Mark, 1957; Conrad and Miller, 1957; Kibler and Johnson, 1961). Oxygen consumption has generally been measured over a relatively short period of time (< 60 min) and when the animals were inactive. In addition, rats used are often fasted and maintained at ambient temperature higher than employed in this study.

We have attempted to measure the metabolism of the rat maintained under standard waveguide cage conditions, spanning the circadian cycle of light/dark photoperiod, food consumption, and physical activity. Our measures of oxygen consumption and carbon dioxide production at times approximate "basal," or resting, levels. At other times during the day/night cycle, however, our measures are considerably higher than those often reported and are in the range consistent with values for active or exercising animals or for animals maintained at low ambient temperatures (Gridgeman and Heroux, 1965; Pasquis et al., 1970; Bedford et al., 1979; Gleeson and Baldwin, 1981).

Differences between the exposed and sham-exposed treatment condition were noted in oxygen consumption, carbon dioxide production, and metabolic quotient for the young but not for the mature animals. This conclusion is supported by both methods of analysis used.

The effects observed in the young animals were less pronounced during the second round of measurements. On an hour-to-hour basis, the mature animals' metabolic measures appear less variable than those of the young. The young animals demonstrate more marked responses to the lights-off condition and generally higher levels on each measure during the nighttime hours, i.e., the active portion of the rats' circadian cycle. This apparent synchronization of metabolic activity with the light/dark cycle has been noted by others investigating the variation of activity, food/water consumption, and energy balance patterns as a function of photoperiod (Siegel, 1961; Zucker, 1971; Besch, 1970; Besch and Woods, 1977).

The apparent greater cage temperature rise of the mature exposed animals and the lower cage temperature rise of the young exposed animals might indicate major differences in thermoregulatory adjustments and/or strategies between the mature and young animals in response to microwave energy absorption. The original intent, however, was simply to monitor cage temperature rise as a check on the adequacy of the airflow rates used. The average temperature differences observed between the exposed animals and their respective sham-exposed controls are on the order of .1-.2 °C which is approaching the resolution with which the temperature measurements were made. In addition to any thermal component attributable to microwave energy absorption, cage temperature rise is a function of the metabolic activity of the animal and, most directly, the airflow rate. The airflow through each of the metabolism cages was roughly the same, and no attempt was made on a daily basis to equalize the airflows exactly. Although a normalization procedure was applied to the air temperature measures to account for variations in airflow rates, how well the cage temperature changes reflect only metabolic endpoints is not clear.

That there were no discernible differences in oxygen consumption and carbon dioxide production in the mature animals at this level of microwave exposure is in agreement with previous research completed by Phillips et al. (1975). They exposed male rats (approx. 430 g) to microwaves at various specific absorption rates corresponding to thermal energy depositions of approximately 27, 40, and 68 cal/min for 30 min and measured postexposure oxygen consumption. The animals receiving 40 and 68 cal/min showed lower levels of oxygen consumption and carbon dioxide production, but no differences were noted for animals receiving 27 cal/min compared to control animals (0 cal/min). The microwave exposure levels used in our study result in a deposition of thermal energy of approximately 1.5-2.0 cal/min throughout the lifetime of an animal, well below that employed by Phillips. The relative contribution of these levels of thermal energy deposition can be placed in perspective if the caloric expenditure of a 400-g rat is taken to be approximately 50 cal/min (cf. Magnen and Devos, 1982).

Under the ambient environmental conditions of temperature, humidity, and airflow, the rate of energy deposition used in this study apparently is not sufficient to produce robust changes in the metabolism of the mature rat exposed to microwave radiation. The changes in the various measures of metabolism observed in the young exposed-animal population and the possibility that these changes may be more pronounced on the first round of measurements are consistent with the fact that the rate of energy absorption (SAR, W/kg) in this waveguide apparatus increases with decreasing body mass.

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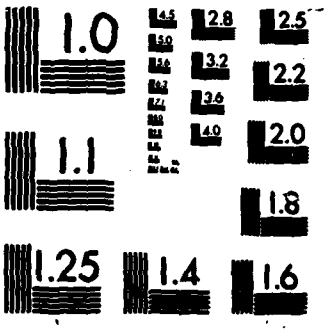

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